

Studies on the Osmophilic Fungus *Wallemia sebi* as an Allergen Evaluated by Skin Prick Test and Radioallergosorbent Test

Tatsuo Sakamoto^a, Atsuo Urisu^b, Masanori Yamada^a, Yoshio Matsuda^c, Kenji Tanaka^d, Shimpei Torii^a

^a Department of Pediatrics and ^d Laboratory of Medical Mycology, Nagoya University School of Medicine, Nagoya;

^b Department of Pediatrics, Fujita-Gakuen Health University School of Medicine, Toyoake;

^c Public Health Research Institute of Kobe City, Kobe, Japan

Abstract. Recently, *Wallemia sebi*, a species of osmophilic fungi, has been abundantly detected in house dust using low water activity media. In this study, allergenic activity of *W. sebi* was assessed by skin prick test and radioallergosorbent test (RAST) in 74 asthmatic patients ranging from 6 to 32 years of age. *Aspergillus fumigatus* and house dust were used for comparison. In skin prick test, *W. sebi* extract, *A. fumigatus* extract and house dust extract elicited positive reactions in 4 (5.4%), 4 (5.4%) and 51 (68.9%) patients, respectively. RAST showed positive results in 14 subjects (18.9%) for *W. sebi* extract, in 8 (10.8%) for *A. fumigatus* extract and in 59 (79.7%) for house dust extract. These results indicated that some asthmatic individuals showed immediate-type hypersensitivity to *W. sebi*, and which means this fungal species may be of importance to atopic diseases as a causative agent.

Introduction

Fungi are able to grow wherever there is an organic matter that serves as a source of food supply, so it is not surprising that they have been considered as a possible causative agent of allergic diseases in humans. But osmophilic fungi were not recognized to be widely distributed in an inhabited environment. The reason is that osmophilic fungi rarely recover from samples for survey because the incidence of environmental molds are usually detected by the use of standard agar media with water activity approaching 1.00 [1], on which media osmophilic fungi grow poorly or not at all [2, 3]. Then, little attention was paid to these fungi as an allergen except the genus *Aspergillus* and the genus *Penicillium*. However, the truth is that many osmophilic fungi can grow well in house dust containing dry organic substances in equilibrium with an atmosphere of normal humidity, while nonosmophilic fungi are restricted to moist substrates and/or abnormally moist atmospheres [1, 4, 5]. Therefore, osmophilic fungi must be found more frequently indoors, if a better method is devised.

Recently, *Wallemia sebi*, a species of osmophilic fungi, has been abundantly recovered from house and mattress dust using low water activity media such as

dichloran glycerol agar medium (DG18) or 64% sucrose malt agar medium (MA64) [2, 4-6]. By our survey of airborne fungi using DG18, *W. sebi* was predominantly found in 15 of 25 indoor samples [unpubl. data]. In addition, the conidium of *W. sebi* has a shape of rough-surfaced sphere with 2.5-3.5 μ m in diameter [7], and so it can reach the respiratory bronchioles [1]. These observations suggest that *W. sebi* may be one of the effective causative agents of human allergies, particularly bronchial asthma. However, allergenic activity of *W. sebi* has not been studied, except Rijckaert's [5] report on the skin reactivity to the fungus.

Our present study reports the results of skin prick test and radioallergosorbent test (RAST) for *W. sebi* extract performed on asthmatic individuals and discusses the etiological importance of the fungus *W. sebi* in atopic diseases.

Materials and Methods

Strains of the Fungi

W. sebi NUM 10116 and *A. fumigatus* NUM 11017 were used for fungal antigen preparation. They were maintained in M40Y agar with the following formulation: malt extract, 20 g; yeast extract, 5 g; sucrose, 400 g; agar, 15 g; and distilled water to 1 liter.

Extract Preparation

W. sebi was cultured in YNB liquid medium composed of 6.7 g of yeast nitrogen base (Difco Laboratories, Mich.) and 600 g of sucrose per liter at 25°C for 3 weeks with gentle shaking. Fungal mats predominantly comprising mycelia were harvested by filtration and defatted with ethyl ether. After air-drying, the mats were homogenized by a Polytron homogenizer (Kinematica, Switzerland) in Coca's solution (1:20 w/v) for 5 min. The homogenate was extracted in the solution for 72 h at 4°C with stirring and centrifuged at 10,000 g for 30 min at 4°C, and then the pellet was discarded. The supernatant was dialyzed against distilled water for 16 h at 4°C and lyophilized.

A. fumigatus was cultured in YNB liquid medium composed of 6.7 g of yeast nitrogen base and 30 g of sucrose per liter at 25°C for 3 weeks in a stationary flask. Extract of *A. fumigatus* mycelial mats was prepared by the same method as used for *W. sebi* extract preparation. Commercially available house dust extract (Torii, Japan) was used.

Subjects

For skin prick test and RAST, 74 asthmatic patients ranging in age from 6 to 32 years (mean $\pm$ SD = 11.7 $\pm$ 4.2 years) were selected randomly from Pediatric Allergy Clinics of the National Chubu Hospital and Nagoya University Hospital on the basis of personal history of bronchial asthma. Fifty-four nonatopic patients (aged 5–28 years, mean $\pm$ SD = 12.0 $\pm$ 3.9 years) who had no personal history of bronchial asthma, allergic rhinitis or atopic dermatitis served as control subjects in RAST.

Skin Prick Test

All subjects were tested by the same investigator and before testing patients were free from treatment except when mentioned. Corticosteroids had been stopped for at least 1 week, antihistamines had been discontinued for at least 3 days, theophyllines and beta-agonists had been suspended for at least 12 h prior to tests. Tranilast and ketotifen had not been administered at least 1 week before tests. The inhalation of DSCG was allowed. Skin prick test was performed on the volar forearm. Fungal extracts diluted to 1% (w/v) in 50% glycerine, and house dust extract diluted to 0.5% were used. Then 0.1% histamine dihydrochloride and 50% glycerine were employed as positive and negative controls, respectively. Reactions were measured after 15 min, and wheal size was determined by averaging the diameters in two dimensions. A reaction was considered positive if it was 3 mm greater than the negative control.

RAST

The procedure of RAST using polyvinyl chloride microplate (Sumitomo Bakelite, Japan) as solid phase was as follows: Each well was first filled with 50 μ l of a solution containing one of the three antigens: *W. sebi* extract (50 μ g protein/ml), *A. fumigatus* extract (20 μ g/ml) and house dust extract (50 μ g/ml) diluted in 0.1 M sodium carbonate buffer (pH 9.8), and incubated at 37°C for 3 h. After washing four times with phosphate-buffered saline (PBS) with 0.05% Tween 20, pH 7.2, unoccupied sites in each well were blocked with 3% human serum albumin (HSA; Calbiochem, Calif.) in 0.1 M sodium carbonate buffer, pH 9.8, for 1 h at room temperature. After washing three times, 50 μ l of test sera diluted 1:2 with PBS was incubated independently in the antigen-coated wells for 3 h at room temperature, except that the sera tested in *A. fumigatus* RAST were diluted 1:8 with PBS. Then washing was done 4 times

to remove unreacted serum. Fifty microliters of ^{125} I-labelled antihuman IgE (Pharmacia Diagnostics, Sweden) containing approximately 20,000 cpm was added and incubated overnight at room temperature. Each well was again washed 4 times and placed in a counting tube, and counted on a gamma counter (ARC-600, Aloka, Japan). These assays were performed simultaneously and in duplicate. Results of the RAST were expressed as percent uptake of ^{125} I-antihuman IgE relative to the total amount (100%) of ^{125} I-antihuman IgE added. The RAST binding over the mean value plus 2 SD of 54 controls was defined as positive.

RAST Inhibition

Each of the inhibiting antigens, *W. sebi*, *A. fumigatus* and house dust, was dissolved at serial concentrations ranging from 0.8 to 100 μ g protein/ml with PBS. This solution was mixed with pooled serum from 5 individuals with highly positive RAST to *W. sebi* and *A. fumigatus* in the ratio of 3:1, and incubated overnight at 4°C. The second step was identical to that described for the RAST. Results were expressed in terms of percent RAST inhibition by the test antigen, which was calculated as follows: $100 \times (^{125}\text{I uptake without inhibition} - ^{125}\text{I uptake with inhibition}) / (^{125}\text{I uptake without inhibition} - \text{background})$.

Results

Skin Prick Test

All of the 74 asthmatics tested showed a positive skin reaction to 0.1% histamine dihydrochloride. Of the 74 subjects tested, 4 patients (5.4%) showed a positive reaction to *W. sebi* extract, 4 (5.4%) to *A. fumigatus* extract, and 51 (68.9%) to house dust extract. Three of 4 patients with positive skin reaction to *W. sebi* were also positive to *A. fumigatus*. The wheal size of the positive reaction to *W. sebi* extract ranged from 3.0 to 4.0 mm (mean $\pm$ SD = 3.50 $\pm$ 0.58 mm), that to *A. fumigatus* extract from 3.0 to 3.5 mm (3.12 $\pm$ 0.25 mm), and that to house dust extract from 3.0 to 13.0 mm (5.05 $\pm$ 2.11 mm).

RAST

Before testing, we demonstrated the specific binding of IgE antibodies to the allergens fixed to the polyvinyl chloride microplate by both the serum dilution test and the RAST inhibition test: In the serum dilution test, RAST binding of *W. sebi*, *A. fumigatus* or house dust linearly diminished with serum dilutions from 1:2 to 1:128 (fig. 1). In the RAST inhibition test, each of the three antigens produced more than 90% inhibition of RAST to itself at a concentration of 10 μ g/mg (see fig. 4).

The results of RAST to *W. sebi*, *A. fumigatus* and house dust in the 74 asthmatic individuals and the 54 nonatopic controls are illustrated in figure 2. Four-

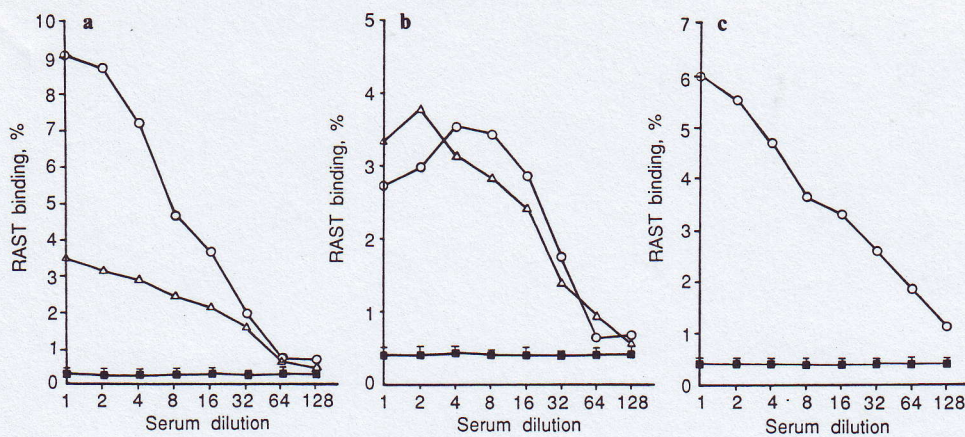


Fig. 1. Effects of serum dilution on RAST binding to *W. sebi* (a), *A. fumigatus* (b) or house dust (c). Sera obtained from 2 asthmatic patients (O, Δ) with positive RAST to solid-phase allergen were serially diluted, ranging from 1:2 to 1:128. RAST results of 5 nonatopic controls are represented as mean plus 2 SD (■).

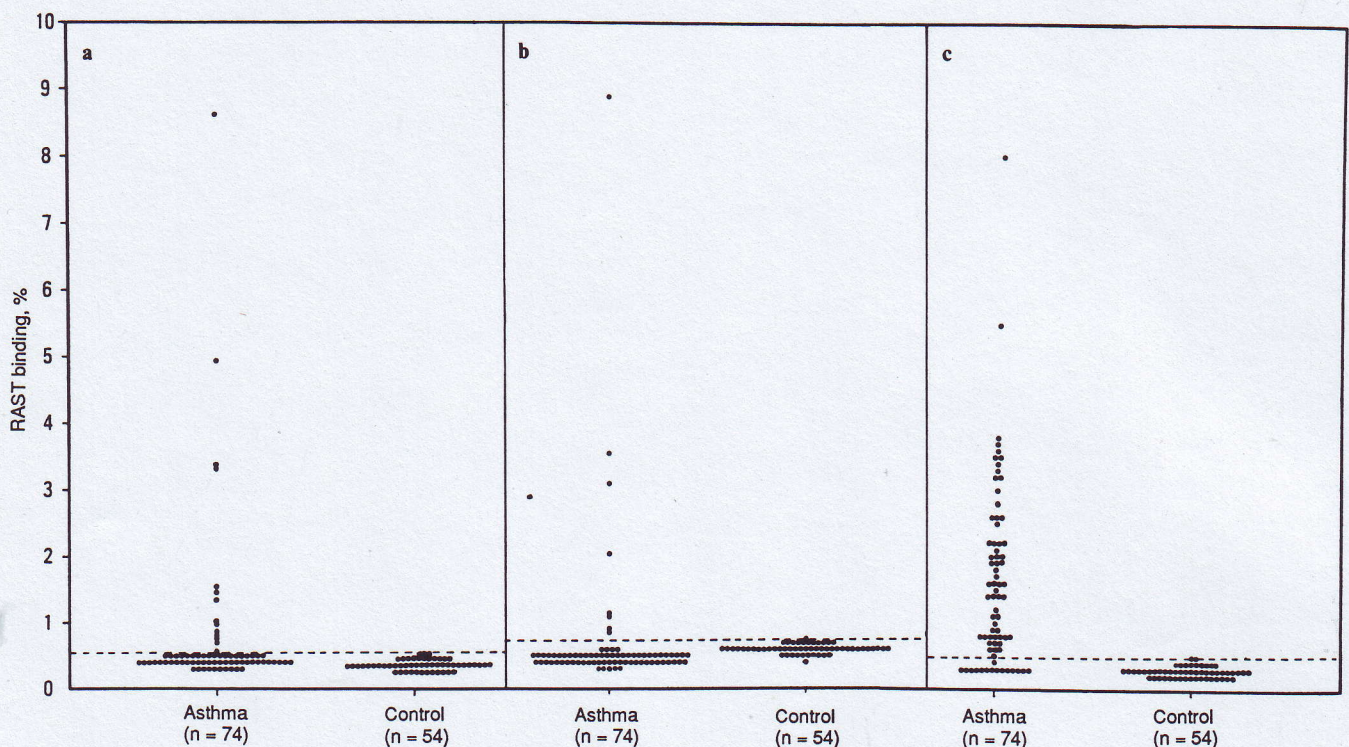
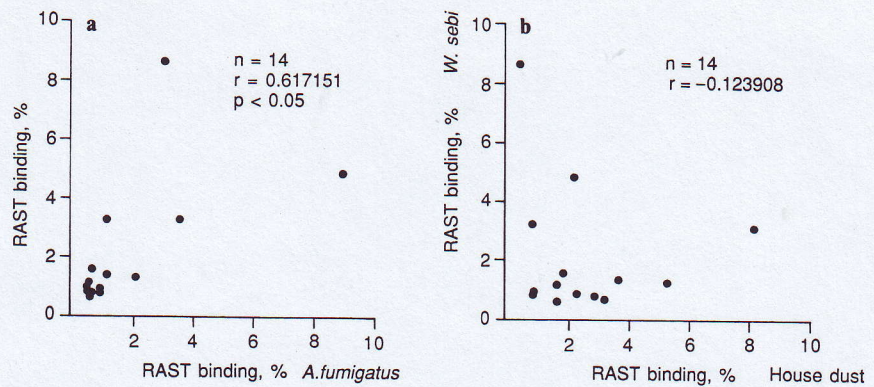


Fig. 2. RAST results for *W. sebi* (a), *A. fumigatus* (b) and house dust (c). RAST results were expressed as percent uptake of ^{125}I -antihuman IgE relative to the total amount (100%) of ^{125}I -antihuman IgE added. The RAST binding over the mean plus 2 SD of controls was defined as positive. Horizontal dotted line represents the lowest positive RAST value. RAST showed positive results in 14 subjects (18.9%) for *W. sebi* extract, in 8 subjects (10.8%) for *A. fumigatus* extract and in 59 subjects (79.7%) for house dust extract in 74 asthmatics.

teen patients (18.9%) were positive in *W. sebi* RAST, 8 (10.8%) in *A. fumigatus* RAST and 59 (79.7%) in house dust RAST. Eight of 14 patients with positive RAST to *W. sebi* also had positive RAST to *A. fumigatus*. The positive RAST value for *W. sebi* extract ranged from 0.70 to 8.65 (mean $\pm$ SD = 2.16 ± 2.27), that for *A. fumigatus* extract from 0.85 to 9.00

(mean $\pm$ SD = 2.72 ± 2.74) and that for house dust extract from 0.47 to 8.05 (mean $\pm$ SD = 1.98 ± 1.33). There was a statistically significant relationship ($r = 0.617151$, $p < 0.05$) between the RAST results of *W. sebi* and *A. fumigatus* (fig. 3a), but not between those of *W. sebi* and house dust ($r = -0.123908$, $p > 0.05$) (fig. 3b).

Fig. 3. Relationship of RAST results between *W. sebi* and *A. fumigatus* (a) and between *W. sebi* and house dust (b). There was a significant correlation between *W. sebi* and *A. fumigatus* ($r = 0.617151$, $p < 0.05$), whereas no significant correlation between *W. sebi* and house dust ($r = -0.123908$, $p > 0.05$).



As indicated in table 1, a highly significant difference ($p < 0.01$ by Fisher's exact test) was obtained in the incidence of positive RAST between the skin prick test positive and negative subjects to *W. sebi*. Similar relationship was obtained for *A. fumigatus*, but no significant relationship for house dust.

RAST inhibition

When tested against *W. sebi* RAST, *A. fumigatus* and house dust exhibited significantly inhibiting activity. *A. fumigatus* and house dust inhibited *W. sebi* RAST by 87.2 and 58.8%, respectively, at the maximal concentration of 25 $\mu\text{g/ml}$ protein (fig. 4a). In contrast, *W. sebi* and house dust inhibited *A. fumigatus* RAST by 32.8 and 29.7%, respectively, at the same concentration (fig. 4b).

Table 1. Correlation between skin prick test and RAST

Allergen	Skin prick test	RAST		Incidence of positive RAST%	Significance
		positive	negative		
<i>W. sebi</i>	positive	4	0	100.0	$p < 0.01$
	negative	10	60	14.2	
<i>A. fumigatus</i>	positive	3	1	75.0	$p < 0.01$
	negative	5	65	7.1	
House dust	positive	42	9	82.4	n.s.
	negative	17	6	73.9	

n.s. = Not significant.

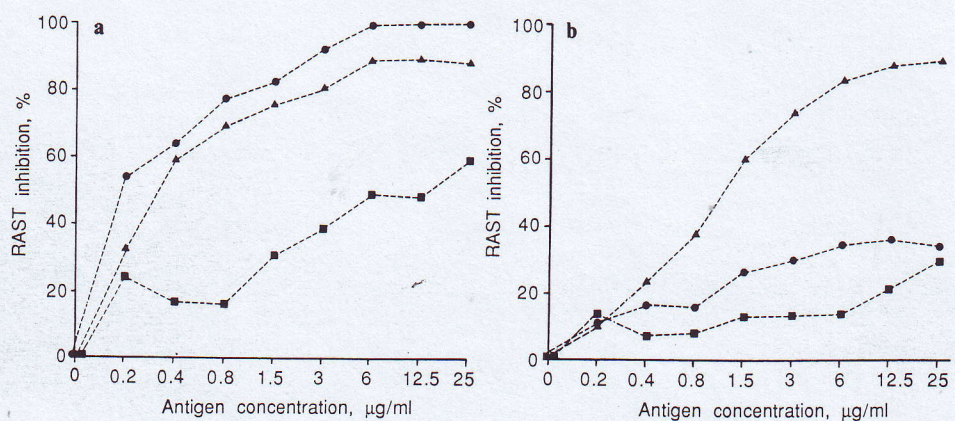
Significance was determined by χ^2 test or Fisher's exact test.

Discussion

For determining allergenic activity in vivo and in vitro of *W. sebi*, we applied skin prick test and RAST,

respectively, to asthmatic individuals. These tests are useful to confirm clinical atopic sensitivity in children, although they might not be enough to clarify the role of a particular allergen as the cause of allergic re-

Fig. 4. Inhibitions of *W. sebi* RAST (a) and *A. fumigatus* RAST (b) by *W. sebi* extract (●), *A. fumigatus* extract (▲) and house dust extract (■). *A. fumigatus* extract and house dust extract inhibited *W. sebi* RAST by 87.2 and 58.8%, respectively, at the maximal concentration of 25 $\mu\text{g/ml}$ protein. *W. sebi* extract and house dust extract inhibited *A. fumigatus* RAST by 32.8 and 29.7%, respectively, at the same concentration.



spiratory symptoms. The results of this study indicated that 4 patients demonstrated immediate-type hypersensitivity to *W. sebi* as evidenced by positive immediate skin reactivity and 14 patients exhibited IgE antibody to *W. sebi* in RAST. The prevalence of hypersensitivity to *W. sebi* by skin prick test and RAST was comparable for that of *A. fumigatus* but less than that of house dust; therefore, allergenic activity of *W. sebi* is probably similar to that of *A. fumigatus* but less than that of house dust.

The RAST inhibition analysis suggested that house dust shared partly allergenic determinants with *W. sebi*. But *W. sebi* might not be the dominant allergen in house dust in this experiment, because there was no correlation between the RAST results of *W. sebi* and house dust.

Positive correlation in RAST results was significantly observed between *W. sebi* and *A. fumigatus*. Furthermore, *A. fumigatus* extract efficiently inhibited RAST for *W. sebi*, and *W. sebi* extract showed also significant but less effective inhibition to RAST for *A. fumigatus*. These results indicated the presence of the cross-allergenicity between *W. sebi* and *A. fumigatus* and that each fungus, however, contained specific allergenic determinants because of the lack of perfect cross-relationship. Cross-reactivity in fungi, irrespective of taxonomic relation, is commonly observed [8-13]. This might be a reason why a clinical manifestation of allergy is difficult to be ascribed to a particular fungal allergen [13]. Therefore, prevalence of a fungus in an environment becomes of paramount importance in determining the role of the fungus as an allergen to atopic diseases. *W. sebi* is probably a predominant species of indoor fungi. This supports that *W. sebi* is an important offending agent in the production of allergic symptoms.

Acknowledgements

We are grateful to Prof. Dr. Kazuyoshi Watanabe, Department of Pediatrics, Nagoya University School of Medicine, for his comments on the manuscript. We also thank Dr. Masano Ueda, and Dr. Hideko Iguchi, Department of Pediatrics, National Chubu Hospital, for their help in testing.

References

- 1 Cole GT, Samson RA: The conidia; in Al-Doory Y, Damson JF (eds): Mould Allergy. Philadelphia, Lea & Febiger, 1984, pp 66-103.
- 2 Lustgraaf Bvd: Xerophilic fungi in mattress dust. *Mykosen* 1977;20:101-106.
- 3 Hocking AD, Pitt JI: Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. *Appl Environ Microbiol* 1980;39:488-492.
- 4 Lustgraaf Bvd, Bronswijk JeHMv: Fungi living in house dust. *Ann Allergy* 1977;39:152.
- 5 Rijckaert G: Exposure to fungi in modern homes. *Allergy* 1981;36:277-278.
- 6 Mori M, Ogami Y, Takahashi T: Microbial contamination of carpet dust in private houses. *J Antibact Antifung Agents* 1985;13:109-117.
- 7 Samson RA, Hoekstra ES, Oorschot CANv (eds): Introduction to Food-Borne Fungi. Baarn, Centraalbureau voor Schimmecultures, 1981.
- 8 Jones HE, Rinaldi MG, Chai H, et al: Apparent cross-reactivity of airborne molds and the dermatophytic fungi. *J Allergy Clin Immunol* 1973;52:346-351.
- 9 Hoffman DR, Kozak P: Shared and specific allergens in mold extracts. *J Allergy Clin Immunol* 1979;63:213.
- 10 Karr RM, Wilson MR, Anticetti VR, et al: An approach to fungal antigen relationships by radioallergosorbent test inhibition. *J Allergy Clin Immunol* 1981;67:194-198.
- 11 Agarwal MK, Jones RT, Yunginger JW: Shared allergenic and antigenic determinants in *Alternaria* and *Stemphylium* extracts. *J Allergy Clin Immunol* 1982;70:437-444.
- 12 O'Neil CE, Reed MA, Hughes JM, et al: *Fusarium solani*. Evidence for common antigenic/allergenic determinants with other Fungi Imperfecti. *Clin Allergy* 1987;17:127-133.
- 13 Salvaggio J, Aukrust L: Mold-induced asthma. *J Allergy Clin Immunol* 1981;68:327-346.

Received: January 18, 1989

Correspondence to: Dr. Tatsuo Sakamoto
Department of Pediatrics
Nagoya University School of Medicine
Nagoya 466 (Japan)