

Rice pollen allergy in Taiwan

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31277

A panel of tests including intracutaneous skin testing (ST), radioallergosorbent test (RAST), immunoblotting and allergen-induced lymphoproliferation was done to study rice pollen allergy in asthmatic children and to characterize the allergens. Of the 312 asthmatic patients skin tested, 29 (9.3%) had positive reactions (wheal ≥ 6 mm) to rice pollen extract at a concentration of 10^{-5} g/mL and the remaining 283 (90.7%) were negative. While eight (34.8%) of the 23 ST-positive patients were also RAST-positive, RAST was negative in all 34 ST-negative patients and 20 normals. Immunoblotting revealed three major allergens, with molecular weights of 16 kD, 26 kD, and 32 kD, respectively. Interestingly, RAST-positive patients showed IgE responses to most allergens but only a few of them had IgG antibodies, while normal controls had stronger IgG responses to the same allergens, particularly to 32 kD, but none had IgE antibody. The preliminary results of rice pollen protein induced-lymphoproliferation were not informative; thus, rice pollen proteins do elicit a specific response in asthmatic children and normals, but its pathogenic role in bronchial asthma needs further study.

INTRODUCTION

Taiwan is a subtropical island, 120.5° east longitude and 24° north latitude. The average temperature in January, the coldest month, is 15.2 °C; in July, the hottest month, 28.4 °C. The relative humidity ranges from 78% (February) to 84% (July). Rice is the most important cereal in Taiwan and rice fields account for 52.6% of the cultivated land area. There are two flowering periods for rice in northern Taiwan, from the middle of June to early July, and from early October to middle of October.

Pollen from ragweed, bermuda, and timothy, etc does not play an important role in the immunopathogenesis of respiratory allergies in children in Taiwan.¹ The flowering seasons of rice coincide with an increase in the occurrence of asthma,^{2,3} and we therefore studied, by using several immunologic variables rice pollen allergy in asthmatic children.

MATERIALS AND METHODS

Patients

A total of 312 asthmatic children was studied, and 20 healthy laboratory personnel were included as controls. Eighty-five percent of patients had elevated serum IgE and/or eosinophilia. Ninety percent were skin test-positive to house dust mite (*D. pteronyssinus*), 50% to molds, 35% to used kapok, 25% to ragweed, and less than 10% to cat or dog danders. The concentration of allergen used for intracutaneous skin testing was 10^{-5} g/mL for mite and 10^{-4} g/mL for other allergens. Antihistamine was prohibited for three days and β_2 agonists were withheld for at least six hours. Sera were collected at the time of skin testing and stored at -20 °C until examined.

Preparation of Rice Pollen Extract

The rice pollen (*Oryza sativa* var. japonica, Tainung 67) was collected from the Experimental Field of Academia Sinica at Nankang, Taipei.

The rice pollen was soaked in distilled water for 24 hours at 4 °C with continuous stirring. After centrifugation at 15,000 g for 20 minutes at 4 °C, the pollen protein content in the supernatant was measured by a modified Lowry's method,⁴ with bovine serum albumin as standard. The supernatant was sterilized by

filtering through a 0.2- μ m membrane filter and then lyophilized. The extract was stored at 4 °C. The same batch was used for skin testing, radioallergosorbent test (RAST), specific lymphoproliferation, and immunoblotting.

Intracutaneous Skin Testings

Skin testing was done by injecting 0.02 mL of rice pollen extract at a concentration of 10^{-5} g/mL into the volar surface of the forearm through a 26-gauge needle and tuberculin syringe to raise a wheal 3 mm in diameter. The result was read at 15 minutes. A wheal size of greater than 6 mm was considered to be positive.

Radioallergosorbent Test (RAST)

The allergen discs were prepared according to the method of Ceska et al,⁵ using allergen discs coupled with allergen of 10^{-3} , 10^{-4} , and 10^{-5} g/mL, respectively. A radioactivity (cpm) of 3-fold or greater than that of a CNBr-activated disc coupled with incubation buffer was defined as positive.

Polyacrylamide Gel Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a vertical slab with 1.5-mm thick polyacrylamide gel (12.5%) according to Laemmli.⁶ The allergen extract was mixed in sample buffer and boiled

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for five minutes. After electrophoresis, the gel was stained with Coomassie brilliant blue R250. Low molecular weight protein standards were used to calibrate the gels for determining the relative molecular weights (Mr) of the electrophoresed components.

Western Blotting

Proteins separated by SDS-PAGE were transferred onto nitrocellulose membrane (Schleicher & Schuell, 0.45 μ m). To block its capacity to further bind protein, the membrane was incubated overnight with 5% bovine serum albumin in Tris-buffered saline (TBS) (10 mM Tris-HCl, 0.5 M NaCl, pH 7.5).

Immunostaining

Following the separation of rice pollen proteins by SDS-PAGE and transferral to nitrocellulose, the membrane was immersed in serum diluted with TBS containing 1% gelatin obtained from each subject at room temperature for 40 hours. The serum was diluted 5-fold for IgE detection and diluted 20-fold for IgG detection. The membrane was then washed in TBS and incubated with ^{125} I-labeled rabbit antihuman IgE or IgG antibodies overnight (Pharmacia, Sweden). The membrane was washed again with PBS containing Tween 20 (1%), air dried, and exposed to X-ray film (Kodak) at -70°C for four hours. The X-ray film was developed and fixed according to the manufacturer's directions.

Allergen-induced

Lymphoproliferation

The lymphoproliferation test was done according to the method of Bradley.⁷ Peripheral blood mononuclear cells (MNC, 2×10^5 cells/well) were incubated in the absence or presence of rice pollen allergen 10^{-4} , 10^{-5} , 10^{-6} g/mL for seven days. Two microCuries ^3H -thymidine (NEN 6.7 Ci/mmol, Boston) was added during the last 18 hours of incubation. The cells were then harvested and radioactivity was counted in a β -counter.

RESULTS

The results of skin testing are shown in Table 1. Among the 312 asthmatic children who were skin tested, 29 (9.3%) gave positive reactions and 283 (90.7%) were negative. All the healthy controls were skin test negative. Most of the skin test-positive asthmatic children are now living or had lived in the countryside.

Table 2 shows the results of RAST. RAST was performed in 23 skin test-positive patients whose sera were available for test. While eight out of 23 (35%) skin test-positive patients also demonstrated a positive RAST, the RAST was negative in all skin test-negative patients and in normal controls. The results of specific lymphoprolifera-

tion induced by rice pollen protein are shown in Table 3. The preliminary data failed to show any significant difference between RAST-positive and RAST-negative patients.

The major allergens of rice pollen were further analyzed by SDS-PAGE and immunoblotting. Figure 1 shows that more than 25 protein bands could be identified by SDS-PAGE. Figure 2 shows that three proteins were capable of eliciting an IgE response in most RAST-positive patients, but not in those with negative RAST. Further, Figure 3 shows that IgG antibodies to those three proteins were much more frequently found in normal controls than in RAST-positive patients, especially to the 32 kD antigen.

Table 1. Results of Intradermal Skin Tests*

Subject	Total No. Tested	Positive (wheal ≥ 6 mm)	Negative (wheal < 6 mm)
Asthmatics	312	29 (9.3%)	283 (90.7%)
Normals	20	0 (0%)	20 (100%)

* Skin testing was done by injecting 0.02 mL of rice pollen extract at a concentration of 10^{-5} g/mL into the volar surface of the forearm to raise a wheal of 3 mm in diameter.

Table 2. Results of RAST*

Subject	No. Tested	RAST	
		Positive	Negative
Asthmatics			
Skin test positive	23	8 (35%)	15 (65%)
Skin test negative	34	0 (0%)	34 (100%)
Normals			
Skin test negative	20	0 (0%)	20 (100%)

* The allergen discs were prepared according to the method of Ceska et al.⁵ using allergen concentrations of 10^{-3} , 10^{-4} , and 10^{-5} g/mL, respectively. A radioactivity (cpm) of 3-fold or greater than that of a BrCN-activated disc coupled with incubation buffer was defined as positive.

Table 3. Rice Pollen Allergen-Induced Lymphoproliferation*

Subject	No. Tested	Control	Allergen, g/mL		
			10^{-4}	10^{-5}	10^{-6}
RAST positive	2	4729	2681†	2665	4073
		5474	3250	6978	8060
RAST negative	2	2158	1134	1515	1655
		2080	2503	4476	4603

* The lymphoproliferation test was done according to the method of Bradley. Peripheral blood mononuclear cells (MNC, 2×10^5 cells/well) were incubated in the absence or presence of rice pollen allergen 10^{-4} , 10^{-5} , and 10^{-6} g/mL for seven days. Two micro-Curies ^3H -thymidine (NEN 6.7 Ci/mmol, Boston) were added during the last 18 hours of incubation. The cells were then harvested and radioactivity was counted in a β -counter.

† cpm.

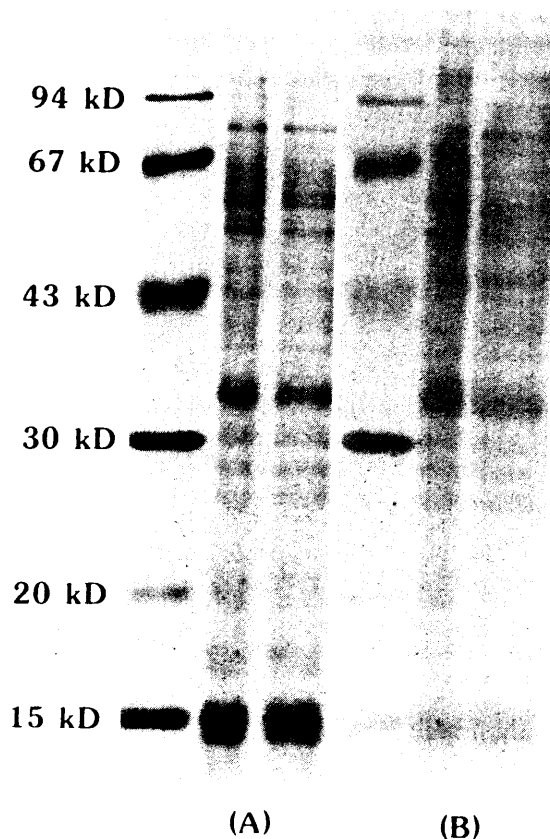


Figure 1. Rice pollen proteins analyzed by SDS-PAGE (A) and those after being transferred to nitrocellulose (B). More than 25 protein bands could be identified.

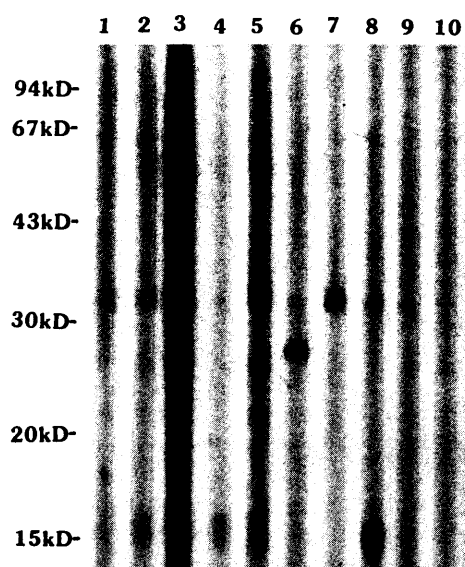


Figure 2. Immunoblotting analysis of rice pollen proteins that elicit IgE antibodies. Lanes 1-8: RAST-positive patients and lanes 9-10: RAST-negative healthy controls. Three proteins of the Mr of 16 kD, 26 kD, and 32 kD elicit strong IgE responses in most patients but not in controls.

DISCUSSION

Airborne pollens and spores show seasonal fluctuations. In Taipei, tree pollen and fern spores present predominantly in the spring and summer, respectively, and in the autumn, the grass pollens, including rice pollen, are the major contributors in the atmosphere.³ Rice is an entomophilous as well as an anemophilous plant. The rice pollen is sized greater than 20 μm but the size of secondary pollen is smaller after going through a process of repetitive dehydration and rehydration. It spreads with the wind and may theoretically enter the airway.

Rice is harvested twice each year in northern Taiwan and the pollinating period is only 1 week. The pollinating period of rice pollen coincides with the peak occurrence of childhood asthma in Taiwan,^{2,3} but only on rare occasions are we able to obtain a clearcut history of exposure followed by acute asthma.⁸ We therefore tried to utilize skin testing, RAST, lymphoproliferative response and immunoblotting to explore the role of rice pollen allergy in the immunopathogenesis of bronchial asthma.

Although in this preliminary study, only 9.3% of the asthmatic children were skin test-positive to rice pollen, the true incidence may be underestimated. Most of the skin test-positive patients had lived or are living in the countryside, but the majority of our patients came from urban areas. Skin testing asthmatic children in country hospitals with rice pollen allergen is now underway.

Because of the lack of well-characterized and purified allergens, a considerable discrepancy often exists between the results of skin testing and RAST. In this study, only 35% of skin test-positive patients showed a positive RAST;^{1,9} however, none of the skin test-negative individuals who were tested with RAST gave positive results so that the RAST was specific in this limited series. The sensitivity and spec-

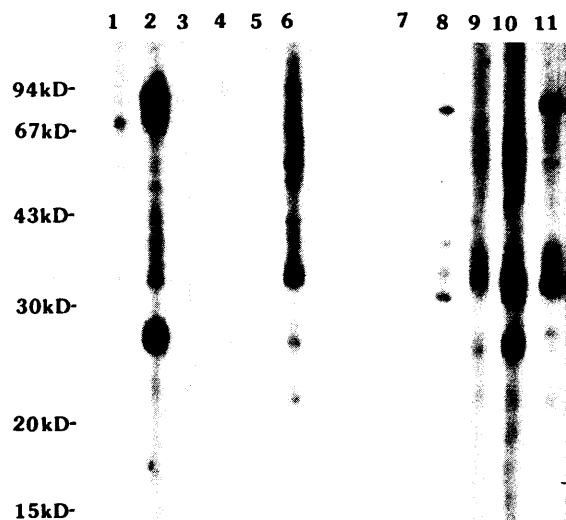


Figure 3. Immunoblotting analysis of rice pollen proteins that elicit IgG antibodies. Lanes 1-6: RAST-positive patients and 7-11: RAST-negative healthy controls. The three major proteins that elicit IgE antibodies in patients also stimulate the production of IgG antibodies in most controls, but in only a few RAST-positive patients.

ificity of RAST were 35% and 100%, respectively. The use of more purified allergen should improve the sensitivity of RAST.

The results of the immunoblotting study are worth noting. More than 25 proteins were identified by SDS-PAGE in rice pollen extract, but only three of them elicited a significant immune response. Their molecular weights are 16 kD, 26 kD, and 32 kD, respectively. More importantly, most of the RAST-positive asthmatic children produced IgE antibodies to these three proteins, but only a few had IgG antibodies. In contrast, none of the RAST-negative healthy normals had IgE antibodies. IgG antibodies, particularly those against 32 kD, were found in most healthy individuals. We have thus shown that a number of rice pollen proteins possess the capacity to elicit a specific immune response, including IgE and IgG. The isotype of antibody produced may be determined by the immunogenetic predisposition of the individuals. Finally, this study suggests again the protective role of

IgG antibody in the immunopathogenesis of allergic disease.¹⁰⁻¹²

Despite the presence of IgE antibodies as demonstrated by skin testing and RAST, no difficulty was found when those patients ate rice during the rice pollen season. No correlation was found among pollen counts, symptom scores, and skin reactivity, although during the past 10 years we did encounter two farmers who presented symptoms of rhinitis and asthma during the rice pollen season for several years.

An extensive search of the literature did not reveal any previous reports that describe the capacity of rice pollen to elicit specific antibody response in atopic and normal individuals. To clarify the role of rice pollen in the immunopathogenesis of respiratory allergies, further studies, particularly a survey of skin reactivity and mucosal challenge among rice farmers, will be done during the next rice pollen season.

REFERENCES

1. Hsieh KH. A study of intracutaneous skin tests and radioallergosor-

- bent tests on 1,000 asthmatic children in Taiwan. *Asian Pacific J Allergy Immunol* 1984;2:56-60.
2. Hsieh KH, Shen JJ. Prevalence of childhood asthma in Taipei, Taiwan and Asian Pacific countries. *J Asthma* 1988;25:73-82.
3. Chen SW, Chien MC. Two-year investigation of the airborne pollen at Nankang, Taipei, Taiwan. *Taiwan J Allergy* 1986;31:33-40.
4. Schacterle GR, Pollack RL. A simplified method for the quantitative assay of small amounts of protein in biologic material. *Anal Biochem* 1973;51:654-655.
5. Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J Allergy Clin Immunol* 1972;49:1-9.
6. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-685.
7. Bradley LM. Cell proliferation. In: Mishell BB, Shügi SM, eds. *Selected methods in immunology*. San Francisco: W. H. Freeman and Co, 1980;158-166.
8. Hsieh KH, Yang YM. Long-term follow-up of asthmatic children. *Acta Paediatr Sin* 1968;9:14-19.
9. Johnsson SGO. Methods for the detection of total and specific IgE antibodies in children. In: Businco L, ed. *Advances in Pediatric allergy*. Amsterdam: Excerpta Medica, 1983;3-13.
10. Evans R, Pence H, Kaplan H. The effect of immunotherapy on humoral and cellular responses in ragweed hay fever. *J Clin Invest* 1976;57:1378-1385.
11. Nagaya H. Long-term effects of conventional immunotherapy in southern California. *Ann Allergy* 1980;44:193-199.
12. Hsieh KH, Lue KH, Chiang CF. Immunological changes after hyposensitization in house dust-sensitive asthmatic children. A review. *J Asthma* 1987;24:19-27.

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