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Sensitization to Sugar Cane Pollen in Okinawan Allergic Children

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SUMMARY **Specific IgE antibodies to sugar cane pollen were investigated in seventy-four Okinawan children who suffered from allergic disorders. Only two (2.7%) with asthma of the 74 patients had specific IgE antibodies to sugar cane pollen as well as house dust and *Dermatophagoides farinae*. However, they have no histories of their symptoms being aggravated when sugar cane flowers bloom. From these results, the sensitization to sugar cane pollen exists in a small number of Okinawan allergic children, however, the low incidence of positivity could reflect lack of exposure to sensitizing doses of sugar cane pollen in these children.**

Sugar cane growing in Japan is limited to Okinawa, and sugar cane cultivation is one of the basic industries in Okinawa. Cane sugar is obtained from juice expressed from sugar cane stalks. Sugar cane flowers bloom from November to January and stalks are harvested in January in Okinawa. Sugar cane pollen has a globular shape, and the mean diameter is 38.6 μm. In Hawaii, the associated foliage, which constitutes a large amount of tonnage, is burned away at harvest time. Thus, a number of allergic patients in Hawaii gave histories of their allergic symptoms being aggravated when they were exposed to smoke from the burning of sugar cane fields.[1] In Okinawa, however, the foliage is left on the ground without being burned, so the Okinawan people including children are not exposed to sugar cane smoke. Lehman[1] reported that many allergic patients in Hawaii previously exposed to the smoke from burning sugar cane developed a positive intracutaneous whealing response. However, it is not known whether sugar cane pollen itself acts as an allergen or not. Thus, we have investigated specific IgE antibodies to an extract of sugar cane pollen by radioallergosorbent test (RAST) in Okinawan children in order to know the sensitization to sugar cane pollen in them.

MATERIALS AND METHODS

Subjects

Seventy-four patients who had been referred for evaluation of probable food or inhalation allergies at the Department of Pediatrics, Okinawa Prefectural Nanbu Hospal comprised the subjects of this study. Informed parental and/or child consent was obtained. They had lived in Okinawa all their lives. The ages of the 43 male and 31 female subjects ranged from 9 months to 15 years (mean 6.3 ± 4.2 years). Forty-seven were found to have asthma, 8 to have atopic dermatitis, 9 to have asthma and atopic dermatitis, 6 to have asthma and allergic rhinitis, 4 to have asthma, atopic dermatitis and allergic rhinitis. The mean of the serum IgE levels for the group was 962.6 ± 1237.1 IU/ml.

Extract of sugar cane pollen

An extract from sugar cane pollen was prepared from collected sugar cane flowers according to the

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method of Markussen *et al.*[2] Briefly, 10 g doses of sugar cane pollen were extracted overnight in 100 ml of solvent, 0.125 M ammonium bicarbonate and 0.015 M sodium azide (pH 7.6). After filtration, the solutions were centrifuged twice for 60 minutes at 50,000 × g. The supernatants were then dialyzed twice against a 50-fold volume of 0.005 M ammonium bicarbonate and 0.015 M sodium azide and then once against water, each for 24 hours at 5°C. A spectra/Por 3, molecular weight cutoff 3,500 membrane tube (Spectrum Medical Industries Inc, Los Angeles, USA) was used for dialysis. Finally, the extracts were freeze-dried and stored at 5°C. Ten grams of sugar cane pollen yielded freeze-dried material. The freeze-dried products were dissolved in a 50:50 mixture of glycerol and saline, with 4% phenol as a preservative, to make 5% (w/v = original amount of pollen/final volume of test solution) test solution.

Activation and coupling procedure

Activation of paper discs and coupling of allergens to activated paper were performed according to the method of Ceska *et al.*[3] Ten g of paper discs (diameter 5 mm) were cut with a punch. The discs were allowed to swell for 30 minutes in 200 ml of water. CNBr solution, 200 ml (5% in water), was added and mixed with a mechanical stirrer for 3 minutes in a water bath at 19°C. NaOH (1 M), 40 ml, was added dropwise to maintain the pH in the range of 10.0 to 10.5. The suspension was immediately poured into 2 l of cold $NaHCO_3$ solution (0.005 M, 4°C). After thorough mixing, the solution was decanted. The washing with 2 l of $NaHCO_3$ solution was repeated five times. The paper discs then were washed four times with 500 ml of acetone (reagent grade, 4°C) and placed on a filter paper in the cold room (4°C) for 3 hours. The drying was continued overnight in a vacuum-desiccator, over $CaCl_2$, at 4°C. The paper discs were then stored at -20°C. No decrease in activity was detectable after 8 months of storage.

The coupling procedure was begun by placing 200 activated discs into a 125 ml siliconized flask containing 20 ml of test solution. The flasks were sealed with parafilm and left on a very slow shaker (about 100 rpm) in a cold room (4°C) for 20 to 24 hours. After coupling, 50 ml of $NaHCO_3$ solution (0.1 M) was added, and the solution was drawn off by inserting a Pasteur pipette connected to a water pump. β-ethanolamine solution (0.05 M in 0.1 M $NaHCO_3$) 50 ml was added and agitated on a horizontal shaker (about 100 rpm) for 3 hours. The solution was drawn off and the paper discs were then washed once with 50 ml of 0.1 M $NaHCO_3$ and three times with 50 ml of acetate buffer (0.1 M, pH 4.0), and twice with 50 ml of "incubation buffer" (0.9% NaCl, 0.05 M phosphate buffer, 0.3% human serum albumin, 0.05% sodium azide, final pH 7.4 at 20°C).

RAST

Serum solution, 50 µl, obtained from allergic patients, diluted 2:3 with incubation buffer, was added to paper discs and agitated on a horizontal shaker (100 rpm) overnight at room temperature. After the incubation, the paper discs were washed three times with 2.5 ml of incubation buffer containing 1% Tween 20. ^{125}I-labelled anti-IgE (40,000 cpm in incubation buffer) 50 µl solution was added and agitated overnight on a horizontal shaker (100 rpm) at room temperature. The paper discs were then washed three times with a 2.5 ml solution of 0.9% NaCl and 1% Tween 20 in water. The tubes were then closed with plastic caps and radioactivity was counted in a γ-counter.

The RAST to egg white, cow's milk, soybeans, house dust, *Dermatophagoides farinae* and Japanese cedar pollen was performed as recommended by the Phadebas RAST test kit (Pharmacia, Uppsala, Sweden).[4] RAST results were scored 0 to 4+ by comparison with serially diluted reference sera (Pharmacia) graded A to D. The reference serum was obtained from patients with sensitivity to pollen of *Betula platyphylla*. The cpm of test serum < the cpm of reference serum D = RAST score 0 (< 0.35 Phadebas RAST units [PRU]/ml); between D and C = 1+ (0.35-0.7 PRU/ml); between C and B = 2+ (0.7-3.5 PRU/ml); between B and A = 3+ (3.5-17.5 PRU/ml); and > A = 4+ (≥17.5 PRU/ml). RAST scores of 2+, 3+ and 4+ (≥0.7 PRU/ml) were recorded as positive.

RESULTS

RAST to food allergens

The RAST scores of the patients varied in each food allergen. Of the 74 patients tested, 15 (20.3%) reacted to egg white, 3 (4.1%) to cow's milk, and 4 (5.4%) to soybeans. However, no patients had RAST score 4 to any food allergens (Table 1).

RAST to inhalant allergens

Of the 74 patients tested, 43 (58.1%) reacted to house dust, and 50 (67.6%) to *Dermatophagoides farinae*, but only one (1.4%) to Japanese cedar pollen (Table 1).

RAST to sugar cane pollen

Of the 74 patients tested, only two (2.7%) reacted to sugar cane pollen. Table 2 shows the clinical data of the 2 patients with positive RAST to sugar cane pollen. Patient 1, a seven-year old boy, had been suffering from mild asthma for 3 years, and his elder sister also suffered from asthma. His serum IgE level was moderate, 1700 IU/ml. He reacted to inhalant allergens

Table 1. RAST to allergens.

Allergens	Numbers of RAST scores 0	1	2	3	4	positive ratio (%)
Food allergens						
Egg white	46	13	11	4	0	15 (20.3)
Cow's milk	61	10	3	0	0	3 (4.1)
Soybeans	60	10	4	0	0	4 (5.4)
Inhalant allergens						
House dust	29	2	25	17	1	43 (58.1)
Dermatophagoides farinae	22	2	5	20	25	50 (67.6)
Japanese cedar pollen	73	0	1	0	0	1 (1.4)

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Table 2. Clinical data of the patients with positive RAST to sugar cane pollen.

	Patient 1	Patient 2
Age (yr)	7	8
Sex	Male	Male
Allergic disorder (severity)	BA (mild)	BA (mild), AR
Serum IgE level (IU/ml)	1700	940
RAST score (PRU/ml)		
Sugar cane pollen	2 (0.8)	2 (1.0)
Egg white	0 (<0.34)	0 (<0.34)
Cow's milk	0 (<0.34)	0 (<0.34)
Soybeans	0 (<0.34)	0 (<0.34)
House dust	3 (3.6)	3 (7.9)
Dermatophagoides farinae	4 (≥17.5)	4 (≥17.5)
Japanese cedar pollen	0 (<0.34)	0 (<0.34)

BA, Bronchial asthma; AR, Allergic rhinitis; PRU, Phadebas RAST unit.

except for Japanese cedar pollen, but not to food allergens. He had a positive RAST to sugar cane pollen, but did not have a history of asthma attacks being aggravated when he was exposed to sugar cane pollen. Patient 2, an eight-year-old boy, had been suffering from mild asthma and allergic rhinitis. His serum IgE level was moderate, 940 IU/ml. He reacted to inhalant allergens except for Japanese cedar pollen, but not to food allergens. He had a positive RAST to sugar cane pollen, but did not have a history of asthma attacks, itching, sneezing, running or blocking of the nose being aggravated when he was exposed to sugar cane pollen.

DISCUSSION

Sugar cane is one of several plants with fibers of dimensions thought to be potentially carcinogenic.[5,6] The burning of the cane rows greatly increases airborne suspended particles that may act as an irritant to the respiratory tract tissue. This irritation could promote increased susceptibility to other carcinogens. Rothschild and Mulvey[7] reported that sugar cane farmers who died with lung cancer had worked for longer periods in the sugar cane farm industry than did those sugar cane farmers in whom lung cancer did not develop. On the other hand, Brooks *et al.*[8] have reported that a slight, not statistically significant, excess risk of lung cancer was observed among participants who reported working in the sugar cane industry, and that tobacco use was the most important cause of lung cancer.

Bagasse is the sugar cane plant residue left after the juice has been extracted. Bagassosis, a relatively benign occupational respiratory disease, has been described in persons exposed to moldy bagasse[9] and has been associated with inhalation of spores of *Thermoactinomyces vulgaris*.[10] Moreover, Lehman[1] reported the allergenicity of sugar cane smoke for the first time. Many allergic patients under his care gave histories of their allergic symptoms being aggravated when they were exposed to smoke from the burning of sugar cane, and they developed a positive intracutaneous

whealing response.[1] In Okinawa, however, the foliage is left on the ground without being burned, so Okinawan people are not exposed to sugar cane smoke. Furthermore, it is not known whether sugar cane pollen itself acts as an allergen or not. In this study, we have investigated specific IgE antibodies to some allergens and an extract of sugar cane pollen by RAST in Okinawan allergic children. Many patients reacted to house dust or *Dermatophagoides farinae* but not to Japanese cedar pollen. Concerning Japanese cedar pollen, our patients had very low positive RAST. Japanese cedars do not grow up naturally in Okinawa, therefore, Okinawan children have no opportunity to be exposed to Japanese cedar pollen. The one patient with a positive RAST to Japanese cedar pollen had been to other prefectures in Japan in which Japanese cedar grows, so he had an opportunity to be exposed to the pollen. Of the 74 patients tested, only two (2.7%) had a positive RAST to sugar cane pollen. They reacted to other inhalant allergens, house dust and *Dermatophagoides farinae*, and had histories of their asthma attacks being aggravated when they were exposed to house dust at home. However, their symptoms did not develop in the season in which sugar cane flowers bloomed.

From these results, the sensitization to sugar cane pollen exists in a small number of Okinawan allergic children; the low incidence of positivity could reflect lack of exposure to sensitizing doses of sugar cane pollen in these children.

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