

# Wild rice, hypoallergenic rice—Immunologic comparison

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## ABSTRACT

Rice is a cereal that is mainly produced and widely consumed in Asian countries including Korea. Several reports have suggested a role of IgE-mediated hypersensitivity in asthma and eczema associated with ingestion or inhalation of rice. In Japan, hypoallergenic rices are used for a substitute of common rice in some atopic patients. We performed this study to identify major allergens of rice and changed allergenicity in cooked and hypoallergenic rice. We purified crude extracts from a variety of rice and analyzed their protein distributions by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Based on UniCAP test and skin-prick test, we selected sera with high sensitivity and analyzed specific IgE binding to rice by immunoblotting. In addition, the inhibition rate among some rice was determined by enzyme linked immunosorbent assay and CAP test. As the result of this study, rice with various origins and polishing levels had no difference in protein band pattern. After cooking, it was difficult to detect protein bands distributed in raw rice; and, even through IgE immunoblot analysis, it was impossible to differentiate between wild and hypoallergenic rice. In addition, both wild and hypoallergenic rice still had IgE binding activity on their remaining protein bands. In conclusion, almost all proteins of rice were excluded or weakened in the process of boiling and IgE binding activity still remained even in hypoallergenic rice.

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A food allergy is an abnormal immunologic response to a specific protein in food. The mechanism of food allergy is characterized by IgE-mediated response causing immediate medical complications and non-IgE-mediated response, which is delayed with symptoms developing after several hours to days.<sup>1</sup> The incidence of food allergy may vary among places and different cultures and races. In Korea, the incidence of food allergy is reportedly as high as 1.5% in a population of adults and ~5–6% in children <3 years of age. The most common allergy-causing foods are eggs, milk, peanuts, beans, flour, and fish.<sup>2</sup> Based on the results of a Korean study of 3320 children with asthma, 11.4% of subjects showed the symptoms of food allergy, and the main culprits were eggs, pork, peaches, sardines, chicken, milk, buckwheat, and crabs in descending order.<sup>3</sup> Allergic reactions are expected to improve by avoiding the food that caused the allergy in most cases; however, elimination from the diet can be difficult or allergic symptoms can persist even after the food is eliminated from the diet.

Rice is a staple food in most Asian countries and rice consumption is increasing in western countries as peo-

ple recognize its nutritional values. There have been several studies on rice allergy, asthma, and severe atopic dermatitis published in Japan. In a move to bring down the incidence of allergic reactions corresponding to rice consumption, hypoallergenic rice was developed in Japan. Rice is an important crop in Korea too, but there have been few studies of rice allergic reactions.

The aim of this study was to examine how antigenic properties of rice vary with the origin of rice, the degree of milling, and the type of cooking and to compare antigenic properties between Korean rices and Japanese rices. This study is intended to lay the foundation for further studies on rice allergy and to prepare a better diagnostic approach in the same area. Despite antigenic effects of rice, it is not easy to eliminate food strictly. Therefore, the development of hypoallergenic rice can be a solution to help reduce the incidence of rice allergies.

## MATERIALS AND METHODS

### Subjects

All subjects were suspected of having food allergies given their clinical data offered by Yonsei University Medical Center and Youngdong Severance Hospital, where they were treated. They were subjected to the UniCAP (Pharmacia, Uppsala, Sweden) and a skin-prick test to ensure their symptoms. The UniCAP value of 2+ was regarded as positive, and a total of 63 inhalant food allergens (Tori, Tokyo, Japan) were used for the test. A wheal is considered positive when its

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size is larger than that of a positive control by measuring the length of its long axis and diameter.

### Rice Crude Extracts

A variety of polished rices produced across Korea were used with a variation in polishing ratio of 30, 50, and 70%. These rices were prepared in four different forms—raw rice, cooked rice, porridge, and thin porridge (miyeum in Korean)—to determine protein distribution of each form. Generally, rice was cooked by adding ~3–5 times as much water as rice. Porridge was cooked with ~6–7 times as much water. The thin porridge was cooked with ~9–10 times as much water. Hypoallergenic rice marketed as an instant cooking form in Japan also was cooked by microwave according to the package directions.

Raw rices were ground into powder after soaking rice in water for 30 minutes using a grinder. Cooked rice was directly ground in a grinder. The grinding process was omitted for porridge and thin porridge. Each test sample was blended with PBS in a ratio of 1:10, and the agitation of the mixture was maintained at 4°C for 48 hours. After the agitation process, the solution was centrifuged in the range of 2000–15,000 rpm for 20 minutes at 4°C. Then, the supernatant was collected and filtered to remove impurities. The protein extracts obtained from test samples were dialyzed for 3 days using a 3500-mw dialysis membrane. The dialysis buffer, distilled water, was changed three times a day. After 3 days of dialysis, extracts were centrifuged at 12,000 rpm for 20 minutes at 4°C. Then, the extracts were filtered, freeze-dried, and kept at –20°C before use.

### Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

According to the Laemmli method,<sup>4</sup> each sample was mixed with loading buffer (60 mM of Tris-HCl, 25% glycerol, 2% SDS, 14.4 mM of 2-mercaptoethanol, and 0.1% bromophenol blue) before heating in boiling water for 5 minutes. Then, the samples were loaded on a 5–13.5% SDS-polyacrylamide gel for electrophoresis (Small mighty; Hoeffer, San Francisco, CA) with a power supply set at 50/180 V. After electrophoresis, gels were placed in Coomassie blue and the protein patterns for allergen were examined and compared.

### IgE Immunoblot

Immunoblot was performed by Towbin's method. Briefly summarized, after SDS-PAGE, the proteins were transferred to nitrocellulose membrane using transfer equipment (by Towbin's method) and then blocked with PBS buffer containing 5% skim milk at room temperature. The blocked membrane was cut

into 3-mm-wide strips. Sera collected from patients with rice allergy were diluted in a ratio of 1:10, and then this pooled serum was applied to each strip and incubated for 16 hours with shaking. After washing three times, anti-human IgE alkaline phosphatase conjugate (Sigma, St. Louis, MO) were added to those strips. Then, protein bands were visualized as protein reacted with 5-bromo-4-chloro-3'-indolylphosphate *p*-toluidine salt/nitro-blue tetrazolium chloride (Promega, Madison, WI).

### Enzyme-Linked Immunosorbent Assay (ELISA) Inhibition

Each protein extract of rice was coated previously onto an ELISA plate for ~16 hours and then pooled sera preincubated with 0 ~ 30 µg/mL of rice protein were applied and incubated for 1 hour. After washing three times, the serum was reacted with anti-human IgE-biotin conjugate and then incubated with streptavidin peroxidase for color reaction. The absorbance was measured at 405 nm to determine allergen activity of test proteins:

$$\% \text{ inhibition} = \frac{\text{uninhibited OD} - \text{inhibited OD}}{\text{uninhibited OD} \times 100}$$

### CAP Inhibition Test

Pooled sera preincubated with 0 ~ 30 µg/mL of rice protein were used for the uniCAP (Pharmacia) test.

## RESULTS

### Protein Pattern Based on the Origin of Rice

SDS-PAGE showed no significant difference in protein distribution between the two varieties of polished white rices produced in Korea. A major 22-kDa band was detected in the two varieties of highly polished white rice with hypoallergenic properties produced in Japan. The two rice varieties produced in the United States and the testing allergens (Tori) used for the skin-prick test also showed a similar protein pattern (Fig. 1).

### Pre- and Postcooking Changes in Protein Distribution between Polished White Rice and Hypoallergenic Rice

Protein patterns were analyzed for regular polished white rice, milled rice with 30% degree of milling, and regular polished white rice after cooking, all of which were produced in Korea. The same analysis also was conducted for cooked highly polished rice, cooked high pressure-treated hypoallergenic rice, and cooked enzyme-treated hypoallergenic rice, all of which were produced in Japan, for comparison. The protein distribution was observed easily in raw rices, but it became

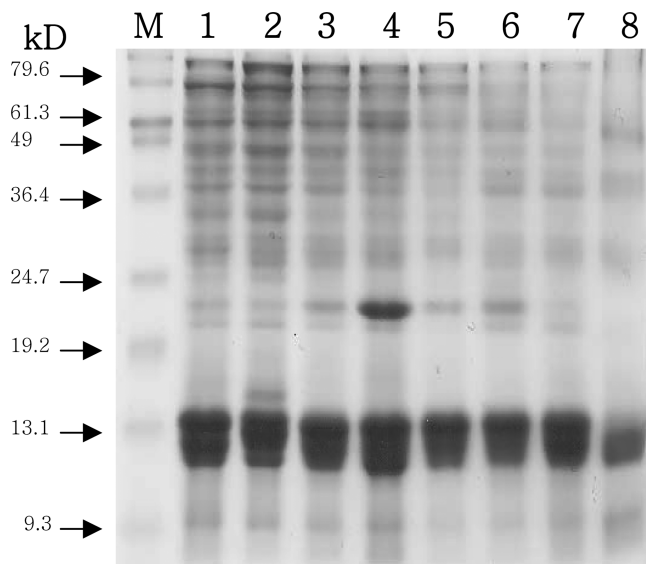


**Figure 1.** SDS-PAGE of raw rices originated from different countries. M, molecular weight marker; lane 1, Korean rice 1; lane 2, Korean rice 2; lane 3, Japanese rice 1; lane 4, Japanese rice 2; lane 5, Japanese rice 3; lane 6, American rice 1; lane 7, American rice 2; lane 8, skin test reagent for rice.

unclear once rices were cooked. There was no significant difference between Korean-origin polished white rice and Japanese-origin highly polished white rice with hypoallergenic properties, except for the 22-kDa band described in Fig. 1 (Fig. 2).

### Changes in Protein Distribution Based on the Type of Cooking

Rices can be cooked in different forms depending on the amount of water. Protein patterns in thin porridge and porridge were compared. Although protein patterns were clearly detected in raw rice, it was difficult to identify protein patterns in all forms of cooked rice including porridge (Fig. 3).

### Changes in Protein Distribution Based on the Degree of Milling

Regular polished white rice is milled to remove 90% of its outer layers and there are other rices produced with 30, 50, and 70% degree of milling; however, there was no significant differences in protein distribution among tested varieties according to SDS-PAGE (Fig. 4).

### Measurement of IgE against Various Rice Allergens

Immunoblot analysis of serum IgE response was performed against allergens of raw polished white rice and milled brown rice with 30% degree of milling, both of which were produced in Korea. The analysis also was undertaken for highly polished white rice, cooked high pressure-treated hypoallergenic rice, and cooked enzyme-treated hypoallergenic

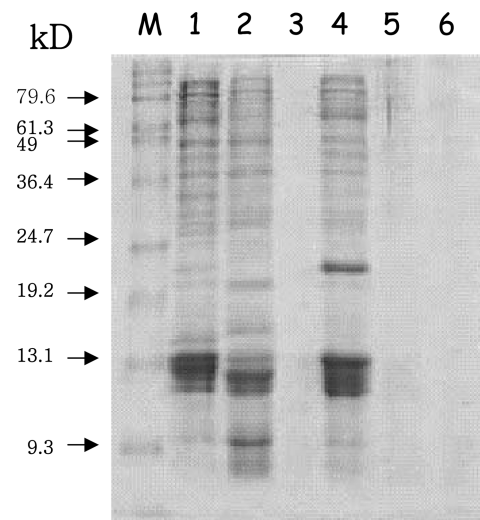


**Figure 2.** SDS-PAGE of rice and hypoallergenic rice. M, molecular weight marker; lane 1, Korean rice; lane 2, partially polished Korean rice; lane 3, cooked Korean rice; lane 4, Japanese hypoallergenic rice; lane 5, cooked Japanese hypoallergenic rice 1; lane 6, cooked Japanese hypoallergenic rice 2.

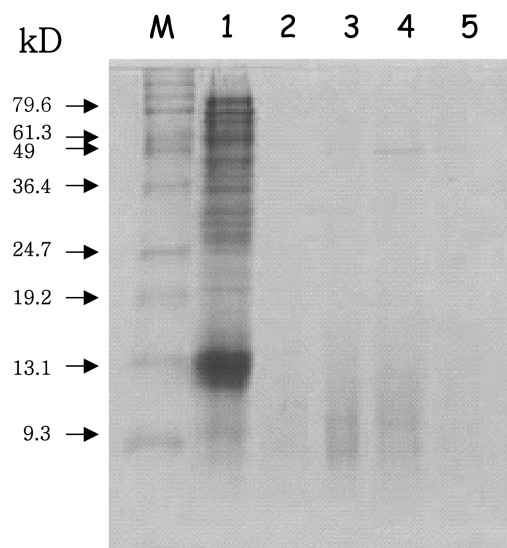


**Figure 3.** SDS-PAGE of raw rice, cooked rice, and porridge. M, molecular weight marker; lane1, Korean rice; lane 2, cooked Korean rice 1; lane 3, cooked Korean rice porridge 1; lane 4, cooked Korean rice porridge 2; lane 5, cooked Korean rice porridge 3.

genic rice from Japan. Sera were pooled from nine subjects whose value of skin test and UniCap serum allergy test was >2 (clinical characteristics presented in Table 1). Although binding proteins with a molecular weight of 16 kDa were detected in all raw rices, no binding protein was observed in cooked rices. It is likely that most allergens detected in raw rices were lost once the rices were cooked, as suggested in SDS-PAGE analysis (Fig. 5).

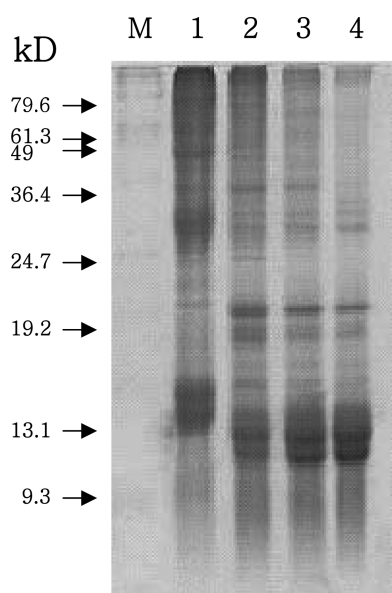


**Figure 4.** SDS-PAGE of various polished Korean rices. M, molecular weight marker; lane 1, fully polished Korean rice; lane 2, partially polished Korean rice 1; lane 3, partially polished Korean rice 2; lane 4, partially polished Korean rice 3.

#### ELISA and CAP Inhibition

The ELISA and CAP inhibition test using sera that were reacted with the testing allergens showed that the allergenic activity was suppressed with the increased concentration of inhibitor (Fig. 6, Table 2).

#### DISCUSSION

Although there were not enough clinical data on rice allergy, most clinicians chose cooked rice for patients sensitized to various food allergens as a substitute diet. In Japan, the aggravating allergic reactions caused by rice in patients with atopic dermatitis have been confirmed through skin-prick test and radioallergosorbent test (RAST), promoting further clinical research on rice

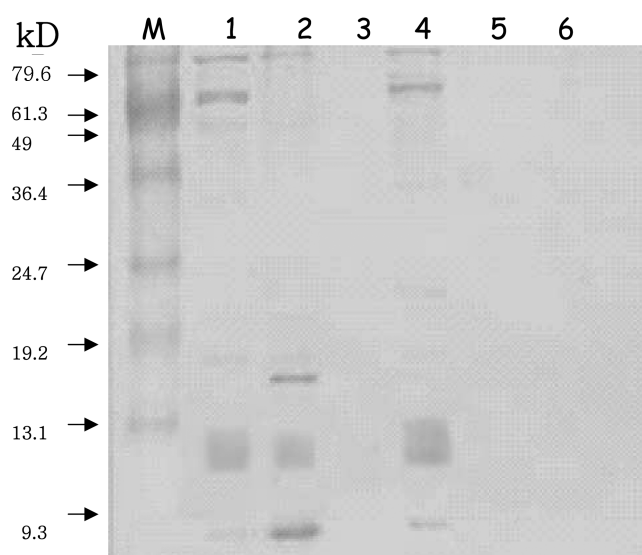


**Figure 5.** IgE immunoblot of rice and hypoallergenic rice. M, molecular weight marker; lane 1, Korean rice; lane 2, partially polished Korean rice; lane 3, cooked Korean rice; lane 4, Japanese hypoallergenic rice; lane 5, cooked Japanese hypoallergenic rice 1; lane 6, cooked Japanese hypoallergenic rice 2.

allergy.<sup>5</sup> Many studies in western countries also reported rice allergy by identifying the major allergens eliciting an IgE response in skin test, bronchial challenge test, contact test with rice powder, histamine release test, and serologic test conducted for patients who suffer from asthma and contact urticaria when they had contact with raw rice.<sup>6</sup> The major food allergens are primarily the water-soluble glycoproteins with molecular weights of 5 ~ 10 kDa, which are resistant to heat, acid, and protease.<sup>7</sup> It is known that the production of allergen-specific IgE antibodies is decreased by 60% when rice is cooked at 100°C for 1 hour.<sup>8</sup> This is the main reason for low clinical reports on rice allergy, but it represents an important factor in

**Table 1 Clinical characteristics of patients**

| No. | Sex | Age (yr) | Diagnosis | TEC (per $\mu$ L) | IgE (mg/dL) | UniCAP Test (kU/L) |      |        |         |       |      |      |        |
|-----|-----|----------|-----------|-------------------|-------------|--------------------|------|--------|---------|-------|------|------|--------|
|     |     |          |           |                   |             | EW                 | Milk | Peanut | Soybean | Wheat | Rice | Corn | Potato |
| 1   | F   | 9/12     | AD        | 5840              | 5000        | 6                  | 5    | 6      | 5       | 6     | 4    | 5    | 3      |
| 2   | M   | 2        | AD, AS    | 1400              | 549         |                    |      |        | 1       |       | 2    | 1    | 2      |
| 3   | M   | 5.3/12   | AD, AS    | 630               | 450         |                    |      |        |         |       | 2    |      | 2      |
| 4   | F   | 7/12     | AD        | 6720              | 3784        | 6                  | 6    | 6      | 6       | 6     | 6    |      |        |
| 5   | F   | 8        | AD, AR    | 430               | 638         |                    |      |        |         |       | 2    |      |        |
| 6   | F   | 8        | AD, AR    | 600               | 1000        |                    |      |        |         |       | 2    |      |        |
| 7   | F   | 9        | AR, AC    | 520               | 248         |                    |      |        |         |       | 2    |      |        |
| 8   | F   | 10       | AS, AR    | 450               | 956         |                    |      |        |         |       | 2    |      |        |
| 9   | M   | 8        | AD, AR    | 800               | 2056        |                    |      |        |         |       | 2    |      |        |

AC = allergic conjunctivitis; AD = atopic dermatitis; AR = allergic rhinitis; AS = asthma; EW = egg white; TEC = total eosinophil counts.

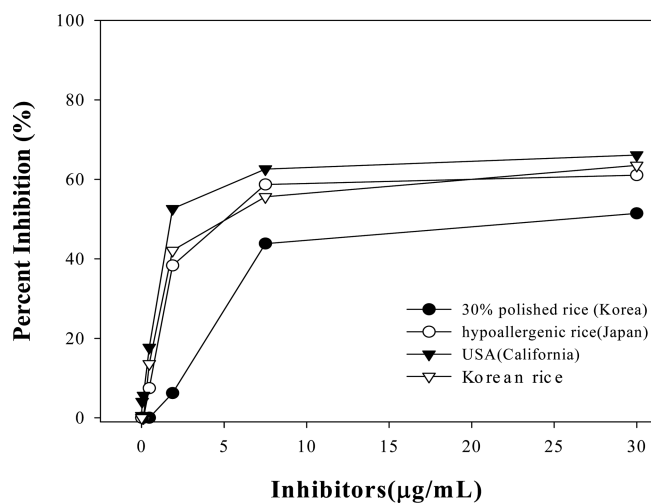


**Figure 6.** Rice inhibition test by ELISA. Crude extract of Korean rice (10  $\mu\text{g/mL}$ ) was coated to the ELISA plate and pooled sera preincubated with 0 ~ 30  $\mu\text{g/mL}$  of rice protein were applied and incubated. After the incubation, the serum prepared was reacted with anti-human IgE and measured at 450 nm to determine the inhibition activity of tested rices. Inhibition rate (%) was calculated by the following formula: % inhibition = (uninhibited OD – inhibited OD)/uninhibited OD  $\times$  100.

**Table 2 CAP inhibition test of less-polished and common rice**

| Rice Crude Extract<br>( $\mu\text{g/mL}$ ) | UniCAP (kUA/L)    |             |
|--------------------------------------------|-------------------|-------------|
|                                            | 30% Polished Rice | Korean Rice |
| 30                                         | 2899              | 2385        |
| 7.5                                        | 4720              | 4646        |
| 1.9                                        | 4770              | 4288        |
| 0.5                                        | 4813              | 4759        |
| 0 (noninhibition)                          | 4617              | 4373        |

organizing a skin test and manufacturing food allergens to be used for placebo-controlled testing. This study provided evidence indicating that it was difficult to detect protein patterns on crude antigen of cooked rices, including a porridge-type mixture, using SDS-PAGE. It is assumed that many proteins are modified and their allergenic properties disappear during the course of cooking. This study also showed that most allergenic activity was destroyed when the rice was cooked. The amount of water used for cooking determines the difference between types of porridge. Thin porridge is prepared for babies who start eating solid food and porridge becomes thicker as babies get used to solid food.

Although clinical research on rice allergy in children is rare, a case report described a boy aged 8 years who experienced rice-induced anaphylaxis and eventually

was diagnosed with eczema based on a positive double-blind, placebo-controlled food challenge. He started a diet that eliminated rice, but showed anaphylaxis again from the smell of cooking rice in the kitchen, leading to the reappearance of symptoms of rice allergy in the bronchial challenge test. His CAP system results were positive, falling into category 4, and immunoblot analysis of IgE revealed that 16- and 27-kDa proteins were major allergens.<sup>9</sup> A study described four infants with rice intolerance such as vomit and shock. Rice was eliminated from the diet for 6 weeks, but the symptoms recurred when rice intake was resumed. The study added that their allergy tests were negative and that thickening of mucous membrane and villus fusion were seen based on pathological changes in intestinal mucosa although the presence of eosinophil in stools was not proven. After the elimination of rice from the diet, the outcome was tracked for 18 months, during which two babies had the same symptoms of rice intolerance when they were fed rice again.<sup>10</sup> Although the study did not prove whether these babies showed IgE-mediated allergic reactions in an immunoblot test, the findings of the study suggested the importance of restricted diet for patients with food allergy. Comparison of rices from Korea, Japan, and the United States showed no difference in protein distribution and specific IgE binding in our results.

Generally, among IgE-reacting allergen bands visualized, allergens made up of 50% of the sera of total patients are identified as major allergens.<sup>11</sup> Earlier studies suggested IgE antibodies in infants with rice allergy reacted to 14 ~ 16-, 26-, 33-, and 56-kDa proteins of rice seed.<sup>12</sup> Among those allergens, rice protein of 16 kDa (RP16 kDa), which was refined by Matsuda, was identified as a major allergen in RAST, RAST inhibition, and histamine release test.<sup>13</sup>

Another study investigated allergen cross-reactions among rice, wheat, corn, Japanese millet, and Italian millet referring to the aforementioned findings. The study reported that these five grains are related according to RAST results and that rice allergens and RP16 kDa and RAST inhibition performed with hulled millets share the same allergenicity, supporting the possibility that IgE binding protein RP16 kDa plays a critical role in eliciting cross-reactions among grains.<sup>14</sup> These findings suggest that a grain chosen for the substitute for rice for patients can aggravate or bring on symptoms of rice allergy if the grain belongs to the Poaceae family as rice. Cross-reacting IgE antibodies in the legume family were, however, rarely proven in a double-blinded test, even when they were suspected.<sup>15</sup>

Japan has played a leading role in the development of new varieties of hypoallergenic rice. The efficacy of hypoallergenic rice depends on how to effectively reduce allergenic properties of rice. Among varieties of

hypoallergenic rice, the HRS-1 (Fine Rice, Shiseido, Japan) produced by enzymic digestion proved effective in infants whose allergic symptoms corresponding to atopic dermatitis had diminished with the elimination of rice from the diet but recurred along with resumed rice feeding. The intake of HRS-1 three times a day for 4 weeks significantly reduced skin troubles and other symptoms, leading to reduced frequency the use of steroid ointment in Japan. There have been three case reports in which the intake of HRS-1 as a substitute for rice aggravated symptoms, and a subsequent IgE immunoblot test revealed a strong IgE binding protein of 52 kDa. Results of these studies suggested the possible role of allergens inadvertently left behind after an anti-allergenic process.<sup>16</sup>

AFT-R1 (Allergen-free Technology Lab., Inc., Tokyo, Japan) is another variety of hypoallergenic rice produced by treating polished white rice with NaOH for 2 hours, neutralizing the treated rice, and rinsing it with flowing water for 24 hours before drying it. SDS-PAGE analysis of heat and salt-soluble proteins extracted from AFT-R1 and polished white rice using NaCl-contained PBS solution revealed no specific protein in salt-soluble proteins of AFT-R1. Most proteins also were destroyed in heat-soluble proteins of AFT-R1, although a part of protein distribution was barely detected. ELISA inhibition test using sera of patients with rice allergy revealed that IgE antibody to AFT-R1 significantly reduced at a dilution of 1:6400, compared with regular rice. In clinical practices, 14 of 15 patients with atopic dermatitis showed favorable outcome after substituting AFT-R1 for regular rice. ELISA test using the serum of the other remaining patient identified the reaction of IgE antibodies to the 60-kDa protein, which was interpreted as ADP(UDP)-glucose-starch glycosyl transferase by N-terminal sequencing.<sup>17</sup>

The varieties of hypoallergenic rice used for this study included two different types of highly polished white rice, high pressure-treated rice, and two different types of enzyme-treated rice that were marketed in Japan with instant packaging designed for cooking in a microwave for 2 ~ 3 minutes. There were no significant differences between Korean-origin regular white rice and Japanese-origin highly polished white rice, according to SDS-PAGE and immunoblot tests. It was difficult to detect protein distribution in three other varieties of hypoallergenic rice because cooked rices were tested for the study. Therefore, the use of raw rice is recommended for further studies involving high pressure-treated rice and enzyme-treated rice. Although no hypoallergenic rice has been imported or produced in Korea, we think that many more clinical

trials are needed before adjusting hypoallergenic rice to patients with allergy in future.

The lack of sera of patients sensitized to rice poses a major obstacle in researching rice allergy in Korea. The effort to identify major allergens in a larger pool of patients showing hypersensitivity to rice seems essential to a successful development of hypoallergenic rice in Korea.

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## REFERENCES

1. Sampson HA. Food allergy. *JAMA* 278:1888–1894, 1992.
2. Sampson HA. Epidemiology of food allergy. *Pediatr Allergy Immunol* 7:42–50, 1996.
3. Kim KE, and Lee KY. Food allergy in children with respiratory allergic diseases. *Pediatr Allergy Respir Dis (Korea)* 5:96–106, 1995.
4. Laemmli UK. Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970.
5. Yamada K, Kishimoto M, Inagaki Y, et al. Study of the role of cereal allergens in atopic dermatitis. *J Jpn Pediatr* 91:888–895, 1975.
6. Lazaun A, Igea JM, Quirce S, et al. Asthma and contact urticaria caused by rice in a housewife. *Allergy* 49:92–95, 1994.
7. Tylor SL, and Lehrer SB. Principles and characteristic of food allergens. *Crit Rev Food Sci Nutr* 36:S91–118, 1996.
8. Kilshaw PJ, Heppell LMJ, and Ford JE. Effect of heat treatment of cow's milk and whey on the nutritional quality and antigenic properties. *Arch Dis Child* 57:842–847, 1982.
9. Fiocchi A, Bouyague GR, Restani P, et al. Anaphylaxis to rice by inhalation. *J Allergy Clin Immunol* 111:193–195, 2003.
10. Cavataio F, Carroccio A, Montalto G, et al. Isolated rice intolerance: Clinical and immunologic characteristics in four infants. *J Pediatr* 128:558–560, 1996.
11. Lownstein H. Quantitative immunoelectrophoretic methods as a tool for the analysis and isolation of allergens. *Prog Allergy* 25:1–62, 1978.
12. Matsuda T, Sugiyama M, Nakamura R, et al. Purification and properties of an allergenic protein in rice grain. *Agric Biol Chem* 52:1465–1470, 1988.
13. Urisu A, Wada E, Kondo Y, et al. Rice protein 16KD—A major allergen in rice grain extract. *Arerugi* 40:1370–1376, 1991.
14. Urisu A, Yamada K, Masuda S, et al. 16-KD rice protein is one of the major allergens in rice grain extract and responsible for cross-allergenicity between cereal grains in the Poaceae family. *Int Arch Allergy Appl Immunol* 96:244–252, 1991.
15. Bernhisel-Broadbent J, and Sampson HA. Cross-allergenicity in the legume botanical family in children with food hypersensitivity. *J Allergy Clin Immunol* 83:435–440, 1989.
16. Ikezawa Z, Tsubaki K, Osuna H, et al. Usefulness of hypoallergenic rice (AFT-R1) and analysis of the salt insoluble rice allergen molecule. *Arerugi* 48:40–49, 1999.
17. Ikezawa Z, Ikebe T, Ogura H, et al. Clinical effect of hypoallergenic rice (HRS-1) in atopic dermatitis. HRS-1 Research Group. *Arerugi* 40:633–642, 1991. □

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