J Korean Med Sci 2000; 15: 387-92
ISSN 1011-8934

A Comparison Study on Allergen Components between Korean (*Arachis fastigiata* Shinpung) and American Peanut (*Arachis hypogaea* Runner)

The prevalence of peanut allergy in Korea is lower than in America. Peanut extract allergens between the two countries have not been standardized. This study was performed to compare the allergenicity of raw Korean and American peanuts with that of roasted peanuts. We prepared the peanut extracts in Korean raw (KP) and roasted peanuts (KRP), and also in American raw (AP) and roasted (ARP) peanuts. We compared the peanut extract allergens of KP, KRP, AP and ARP in vitro with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunoblotting, T-cell proliferation assay and skin prick test with sera from peanut-allergic patients. SDS-PAGE and Western blotting demonstrated four allergenic extracts, numerous bands that displayed a high prevalence of IgE binding. IgE-binding bands were at 64, 36 and 17 kDa. Western blot inhibition revealed that either KP or AP could almost completely inhibit the reactivity of the other extract. There were no differences between T-cell proliferation assay and skin prick test. In conclusion, this investigation showed no different allergic components in both raw and roast extracts of Korean and American peanuts.

Key Words: *Peanut Allergy; Peanut Allergens; Food Hypersensitivity*

Chun-Wook Park, Gyeong-Il Kim, Cheol-Heon Lee

Department of Dermatology, Hallym University College of Medicine and Institute of Medical Sciences, Chuncheon, Korea

Received: 13 March 2000
Accepted: 4 May 2000

Address for correspondence
Chun-Wook Park, M.D.
Department of Dermatology, Kangnam Sacred Heart Hospital, Hallym University, 948-1, Daerim 1-dong, Yeongdeungpo-gu, Seoul 150-071, Korea
Tel: +82.2-829-5221, Fax: +82.2-832-3237
E-mail: knderma@netian.com

*This work was supported by the Institute of Medical Sciences of Hallym University.

INTRODUCTION

Peanut allergy is a significant health problem because of the potential severity of the allergic reaction, life-long nature of the allergic hypersensitivity, and ubiquitous use of peanut products (1). Death due to an anaphylactic shock by peanuts is not uncommon. Assem et al. (2) showed that approximately 30% of all adverse reaction to food in patients with atopic dermatitis is due to peanut. The incidence of allergic reactions to peanuts in Korea is lower than in America. Anaphylactic shock by peanuts has not been reported in Korea. Peanut allergens are relatively heat-stable and are thus present in both raw and roasted peanuts. But some authors suggest that heating could influence the potency of peanut allergens (3). This study examined the immunologic nature of peanut allergy, and compared allergenicity between Korean and American peanuts. Additionally, the reactivity of raw and roasted peanuts allergens was compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblot studies, T-cell proliferation assays and skin prick tests.

MATERIALS AND METHODS

Human sera

Sera from 10 patients with peanut hypersensitivity reactions were used for study. These sera were kindly provided by Dr. Wesley Burks. Sera from five subjects with atopic dermatitis with a negative skin test reaction to peanut served as controls.

Peanut

In general, there are four types of peanuts, which are Runner, Virginia, Spanish and Valencia type. *Arachis fastigiata* Shinpung, one of the most popular peanut in Korea, and a variety developed from crossing Spanish type (Ped 393-6-3-2-2-3-1-2) and Virginia type (Florigiant)/Japanese variety (Chibahandachi) was used in this

Korean raw peanut study. Southeast runner type peanut (*Arachis hypogaea* Runner) which have become the dominant type due to its introduction in the early 1970s was used as the American raw peanut. For roasted peanut study, raw peanut still in the shell was heated for 10 min at 121-149℃.

Peanut extracts and protein content

Shelled peanuts were briefly blanched with water to moisten the peanut hulls for easy removal. Hulled peanuts were then ground in a mortar and pestle or in a food processor to a fine powder. 3-5 grams of powdered peanuts were then extracted with petroleum ether in a Soxhlet extractor for 4 hr in a well-ventilated chemical hood. Heating mantle was set at 37℃ for ether vaporization; cold water to condense ether; reflux took place about every 10-15 min; ether was a pale yellow color when peanuts were defatted. Ether extracted peanuts were allowed to dry until no residual ether odor was noticeable; 1:20 w/v extracts were then prepared in 1 M/L NaCl to 20 mM/L sodium phosphate (pH 7.0) for overnight at 4℃. The extract was isolated by centrifugation at 20,000 g for 60 min at 4℃.

The bicinchoninic acid (BCA) technique (Pierce Chemical Co, Rockford, IL, U.S.A.) was used for protein determination following the manufacturer's instructions with bovine serum albumin (BSA) as a standard.

SDS-PAGE and Western blotting

SDS-PAGE was carried out with a 4-20% polyacrylamide separating gel and a stacking gel of 4%. Twenty microliters of a 1 mg/mL solution of each fraction were applied to each well. Electophoresis was performed for 90 min at 0.030 A per gel (Novex, San Diego, CA, U.S.A.) for the 8 cm by 8 cm gels. To assure proper protein separation and visualization, colloidal blue stain (Novex) was done on gels. Proteins were transferred from the separating gel to a nitrocellulose membrane in a transfer buffer (Tris-glycine) with 0.05% SDS and 40% methanol. The procedure was done in a transblot apparatus (Novex) for 2 hr (0.250 A). After removal from the transblot apparatus, the nitrocellulose was placed in Tris buffered saline-Tween 20 with 1% BSA as a blocking solution for 1 hr. The nitrocellulose blot was incubated with the pooled peanut-sensitive IgE serum (1:20 dilution) for 2 hr at room temperature with rocking. After another wash step, they were incubated 2 hr with horse anti-human IgE labeled with iodine 125 (Santofi, MN). Autoradiographic detection of IgE binding was done by exposing X-OMAT AR x-ray film (Eastman Kodak, Rochester, NY, U.S.A.) to the membrane at 70 ℃. Exposure times was usually overnight.

Western blot inhibitions

KP and AP (20 μg protein of each extract) were fractionated by SDS-PAGE and transferred onto nitrocellulose membranes. Concentrations of either KP or AP (20 μg/mL) and the sera pool were incubated with each blot. The strips were washed, radiolabeled anti-IgE was added, and the strips were incubated overnight. These strips were washed again, and autoradiography was performed for one day. Degree of inhibition was determined by visual inspection by two independent observers.

T-cell proliferation assay

The peripheral blood lymphocytes (PBL) of three peanut sensitive patients were isolated from whole blood using standard procedures for Ficoll Hypaque underlay. Three patients had a positive immediate skin prick test to peanut and either a positive food challenge or a convincing history of peanut anaphylaxis (laryngeal edema or hypotension). Cells were washed and suspended in media at a concentration of 4×10^6 cell/mL. One mL aliquots were placed in 24 well tissue culture plates and stimulated with 50 μg/mL of crude peanut extract (CPE) in order to establish peanut specific cell lines. CPE-specific T-cell lines at 2×10^5 cells/well were then stimulated with either media alone, KP (50 μg/mL), AP (50 μg/mL) or rice extract (50 μg/mL) and allowed to proliferate for six days. On the last day the cells were incubated with [^{3}H]-thymidine (1 μCi/well) for 6-8 hr and then harvested onto glass fiber filters (Packard, Meriden, CT, U.S.A.). T-cell proliferation was estimated by quantitating the amount of [^{3}H]-thymidine incorporation into cellular DNA. [^{3}H]-thymidine incorporation was expressed as a stimulation index which is defined as the fold-increase above media-treated cells.

Skin prick test

The ability of KP or AP to elicit the IgE mediated degranulation of mast cells was evaluated using skin prick tests in peanut allergic individuals. Three individuals meeting the criteria for peanut allergy (convincing history or positive food challenge) and a non-allergic control patients were selected for testing. AP and KP were tested. Twenty μL of the test solution were applied to the forearm of the volunteer and the skin beneath pricked with a sterile needle. Testing was started at the lowest concentration (1 μ/mL) and increased ten fold each round to the highest concentration or until a positive reaction was observed. Mean diameters of the

wheal were measured and compared to the negative control. Histamine was used as the positive control.

RESULTS

Protein concentrations

On analysis of peanut extracts, Korean raw and roasted peanut extracts contained protein concentrations of 6.2 and 8.1 mg/mL, respectively. In American raw and roasted peanut extracts, 7.8 and 12.5 mg/mL, respectively (Table 1).

SDS-PAGE

The Korean and American peanut extracts were very similar with the darkest staining bands having apparent molecular weights of 60-65, 38-48, 28-30, 15-18 kDa. Although there were a higher number of proteins and more distinctive bands in American extracts (Fig. 1).

Western blotting

Western blot analysis revealed multiple allergen bands diffusely scattered through a wide range of molecular weights. In the Korean and American peanut lanes, apparent molecular weights of 64, 36, 17 kDa can be clearly identified. In American peanut extracts, stronger binding to 17 kDa and 200 kDa bands was evident (Fig. 2).

Table 1. Skin prick test* results of peanut allergic individuals and control

Patient	KP†	AP‡	Histamine	PBS
1	20×24	23×25	4×4	ND
2	10×9	11×8	4×4	ND
3	6×6	7×7	4×5	ND
Control	ND	ND	4×5	ND

*The reaction to each allergen is expressed as the wheal diameter (millimeters) measured in two perpendicular reactions.
†The concentration of KP used was 20μg/mL.
‡The concentration of AP used was 20μg/mL.
ND, no reaction detected; PBS, phosphate buffered saline

Western blot inhibition

KP was inhibited by both homologous (KP) and heterologous (AP) inhibitors (Fig. 3). AP immunoblot inhibition demonstrated similar results, with almost equal inhibition by the homologous (AP) and heterologous (AP) inhibitors. These studies indicate that the allergens in the KP and AP shared IgE-binding sites.

T-cell proliferation assay

The ability of AP and KP to stimulate the proliferation of CPE-specific T-cell lines isolated from the peri-

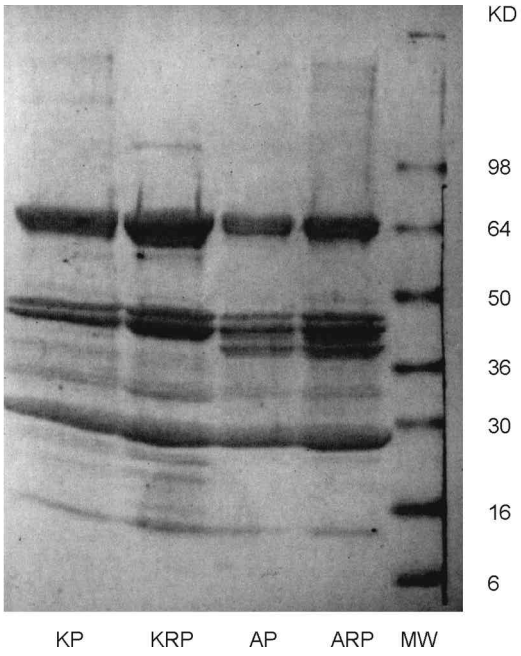

Fig. 1. An SDS-PAGE gel of the crude peanut extract proteins.

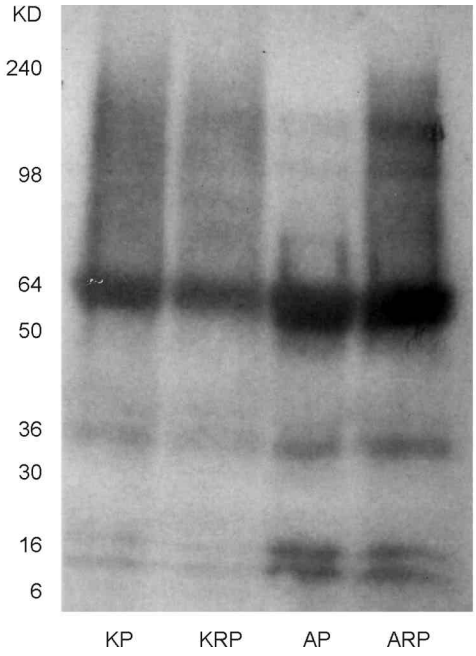

Fig. 2. An autoradiogram of the SDS-PAGE-separated extract proteins immunoblotted with a pooled serum sample from peanut-allergic subjects.

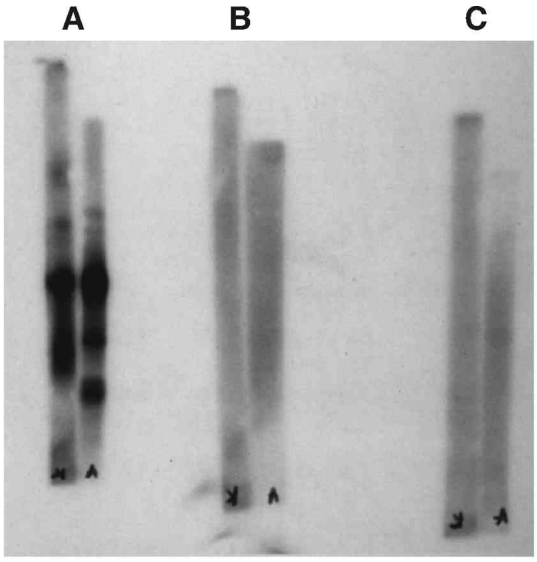

Fig. 3. Western blot inhibition. KP is inhibited by both homologous (KP) and heterologous (AP) inhibitors. Lane A represents Western blot. Lane B and C: Results after absorption of KP and AP extracts.

pheral blood of allergic individuals was measured in response to media, AP, KP, and crude rice extracts (negative control) (Fig. 4). The stimulation index (SI) for individual T-cell lines stimulated with CPE ranged from 10 to 14 in KP and from 11 to 15 in AP when compared to either media or crude rice extract (negative controls) treated cells. These results indicate that the allergen retains its biological activity to stimulate T cells from peanut allergic individuals.

Skin prick test

It was found that AP and KP were able to elicit a positive skin prick reaction in all peanut sensitive patients tested. All three peanut allergic volunteers reacted with KP and AP. The non-allergic individual did not have a positive reaction to either preparation (Table 1). The size of the wheal produced by KP was always slightly smaller than that produced with AP.

DISCUSSION

The identification and the characterization of allergens are critical to our understanding of IgE-mediated diseases. Biological properties of allergenic proteins include their ability to bind serum specific IgE, elicit a positive prick skin test, and stimulate T-cell proliferation from sensitive individuals. In the past several years, multiple

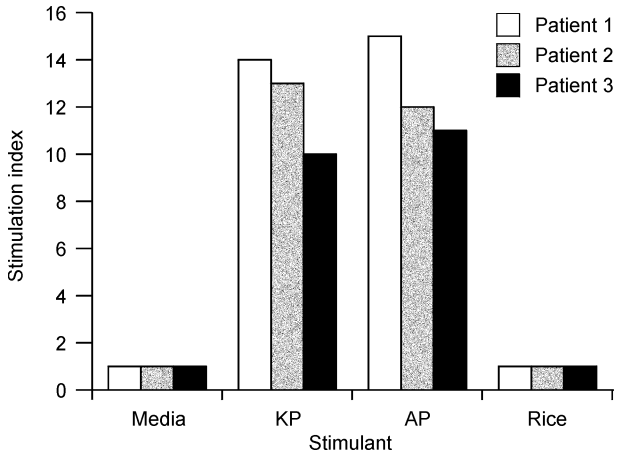

Fig. 4. The stimulation index for individual T-cell lines stimulated with the crude peanut extracts in KP and AP.

allergens that stimulate IgE production have been identified to cause IgE-mediated disease in man. Also, significant work has been done to identify and purify inhaled allergens from a wide variety of sources, including pollens, dust mites, animal danders, insects and fungi. In comparison, very little work has been done on food allergens that are known to cause IgE-mediated diseases.

The reasons why peanut allergy is such an important area of research and clinical service development are clear-cut. It starts early in life, is usually severe and does not resolve (4). Recently, the incidence of peanut allergy has tended to increase (5). A recent population survey in the U.S.A. suggests that approximately 1.1% of the population are allergic to peanuts (6). In a French study of 54 newborn infants (less than 11 days) and 71 babies aged between 17 days and 4 months old, 8% of both groups had positive skin prick tests to peanut, implying sensitization in utero or soon after birth (7). Also, peanut is the most common cause of food-related anaphylaxis and food-related anaphylactic deaths (8, 9).

Because of the prevalence and severity of peanut allergy, several studies have examined the possible major and minor allergens in peanut. Burks et al. identified two major allergens, *Ara h* I and *Ara h* II, with molecular weights of 63.5 and 17 kDa, respectively (10, 11). Some authors showed the occurrence of 16 IgE-binding proteins in peanut extract with a major IgE-binding glycoprotein, the so-called Concanavalin A-reactive protein of approximately 66 kDa (12). Furthermore, peanuts contain a lectin, peanut agglutinin (PNA) which has been demonstrated to bind IgE (13) and possesses the ability to induce proliferation in bovine lymphocytes (14). In our study of Korean and American raw and roast extracts, significant peanut specific IgE antibody binding on the peanut immunoblot was found at 64, 36, 17 kDa regard-

less of the source. The molecular weights of 64, 17 kDa corresponds to *Ara h* I, *Ara h* II, respectively. The 36 kDa bands could be either proteolytic products of either *Ara h* I or *Ara h* III but without 2 D gel with blot protein sequence we can not say which it is. The most likely origin of the 200 kDa band is the natural tendency toward trimerization of *Ara h* I. Heat may influence the allergenicity of some food (3). Humans do not generally consume raw peanuts, so roasted peanuts are a logical choice of extract material. In our study raw and roast extract using western blot showed no difference in allergen components. And, by measuring the ability of AP and KP to stimulate the proliferation of CPE-specific T-cell lines isolated from the peripheral blood of allergic individuals, we know that the allergen retains its biological activity to stimulate T cells from peanut allergic individuals.

The peanut is noteworthy for its thermal stability, maintaining its allergenicity after roasting at 145℃ for 1 hr. But Barnett et al. (3) showed that roasting of peanuts reduces the protein composition, suggesting that extracts from roasted peanuts could show less bands on SDS-PAGE than in peanuts. In our SDS-PAGE and immunoblot analysis, there was no significant difference in protein between the two extracts.

In America, approximately 125 peoples die each year in the U.S.A. to peanut-induced anaphylaxis (15). The prevalence of peanut allergy or anaphylaxis in Korea is unknown because unlike many other disorders, there is no requirement to report such reactions to any national registry. Of course, there are some differences in the prevalence of peanut allergy between countries. The low prevalence of peanut allergy in countries such as Indonesia, where groundnut (peanut) is a part of the staple diet, may be due to the presence of coexisting factors that protect against allergy (16). Different populations and nationalities may consume more of certain foods, and increased exposure may result in increased prevalence of that specific food allergy. In the U.S.A. peanut is one of the most common food allergies. All Americans ingest several tons of peanuts daily (17). By contrast, in Scandinavia, where fish consumption is high, the prevalence of allergic reactions to codfish has increased (15). In Korea, only 0.72 kg of peanut was consumed per individual for 1 year (18). Low peanut consumption may relate to low incidence of peanut allergy. Our study shows no significant difference between Korean and American extracts. Difference in the prevalence of peanut allergy could be explained in different ways. First, diverse food habits between populations, for instance high-peanut consumption in the U.S.A. compared to Korea. Second, genetic backgrounds could also play a role in the development of specific antibodies to an antigen such as peanut allergen. Currently in Korea, the young generations like to eat Western food including peanut butter, nut and foods containing peanuts. Although there are no reports of peanut allergy or anaphylaxis yet in Korea, peanut allergy may be a significant problem in the future.

In conclusion, this study confirms that there is no difference in allergen components between Korean and American peanuts regardless of being raw or roasted.

REFERENCES

1. Burks AW, Cockrell G, Stanley JS, Helm RM, Bannon GA. *Recombinant peanut allergen Ara h I expression and IgE binding in patients with peanut hypersensitivity. J Clin Invest 1995; 96: 1715-21.*
2. Assem ES, Gelder CM, Spiro SG, Baderman H, Armstrong RF. *Anaphylaxis induced by peanuts. BMJ 1990; 300: 1377-8.*
3. Barnett D, Baldo BA, Howden ME. *Multiplicity of allergens in peanuts. J Allergy Clin Immunol 1983; 72: 61-8.*
4. Hourihane JO. *Peanut allergy–current status and future challenges. Clin Exp Allergy 1997; 27: 1240-6.*
5. Sampson HA. *Epidemiology of food allergy. Pediatr Allergy Immunol 1996; 7(9 Suppl): 42-50.*
6. Sicherer SH, Munoz-Furlong A, Burks AW, Sampson HA. *Prevalence of peanut and tree nut allergy in the US determined by a random digit dial telephone survey. J Allergy Clin Immunol 1999; 103: 559-62.*
7. Pumphrey RS, Stanworth SJ. *The clinical spectrum of anaphylaxis in north-west England. Clin Exp Allergy 1996; 26: 1364-70.*
8. Hatahet R, Kirch F, Kanny G, Moneret VDA. *Sensitization to peanut before 4 months-based upon 125 cases [English abstract]. Revue Francaise d'Allergologie et d'Immunologie Clinique 1994; 34: 377-81.*
9. Sampson HA, Mendelson L, Rosen JP. *Fatal and near-fatal anaphylactic reactions to food in children and adolescents. N Engl J Med 1992; 327: 380-4.*
10. Burks AW, Williams LW, Connaughton C, Cockrell G, O'Brien T, Helm RM. *Identification and characterization of a second major peanut allergen, Ara h II, with the use of sera of patients with atopic dermatitis and positive peanut challenge. J Allergy Clin Immunol 1992; 90: 962-9.*
11. Burks AW, Williams LW, Helm RM, Connaughton C, Cockrell G, O'Brien, T. *Identification of a major peanut allergen, Ara h I, in patients with atopic dermatitis and positive peanut challenges. J Allergy Clin Immunol 1991; 88: 172-9.*
12. Barnett D, Howden ME. *Partial characterization of an allergenic glycoprotein from peanut (Arachis hypogaea L.). Biochim Biophys Acta 1986; 882: 97-105.*
13. Burks AW, Cockrell G, Connaughton C, Guin J, Allen W, Helm RM. *Identification of peanut agglutinin and soybean trypsin inhibitor as minor legume allergens. Int Arch Allergy*

Immunol 1994; 105: 143-9.

14. Nagi AM, Babiuk LA. *Peanut agglutinin (PNA): binding and stimulation of bovine intestinal and peripheral blood leukocytes. Vet Immunol Immunopathol 1989; 22: 67-78.*
15. Burks W, Bannon GA, Sicherer S, Sampson HA. *Peanut-induced anaphylactic reactions. Int Arch Allergy Immunol 1999; 119: 165-72.*
16. Ewan PW. *Prevention of peanut allergy. Lancet 1998; 352: 4-5.*
17. Bock SA, Atkins FM. *The natural history of peanut allergy. J Allergy Clin Immunol 1989; 83: 900-4.*
18. Korea Rural Development Administration. *Inclination and planning of peanut production. Nat Crop Exp Station Report 1996; 7-8.*