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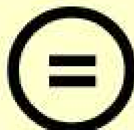

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Evaluation of γ-Irradiated Peanut Extract as a Proper Immunogen for Immunotherapy in Murine Model of Peanut Allergy

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의 학 과

오 세 조

Evaluation of γ-Irradiated Peanut Extract as a Proper Immunogen for Immunotherapy in Murine Model of Peanut Allergy

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- ABSTRACT -

Evaluation of γ-Irradiated Peanut Extract as a Proper Immunogen for Immunotherapy in Murine Model of Peanut Allergy

Background and objectives: Peanut allergy is one of the most serious forms of IgE-mediated food hypersensitivity, and gamma irradiation has been applied widely for the preservation of food. Previous studies indicated that the capacity of IgE to binds to ovalbumin (OVA) and ovamucoid (OM) was profoundly reduced after treatment with a combination of gamma irradiation and other processing treatments, and that this reduction in binding capacity was coupled with reduced OVA and OM skin reactions on skin prick tests. Therefore, this study was conducted to evaluate the feasibility of using the gamma irradiation technique to reduce peanut allergies by observing the changes that occur in the allergenecity and antigencity of gamma irradiated peanut proteins.

Materials & Methods: 1) The physicochemical and imunochemical properties of gamma-irradiated peanuts - Peanut extract that had been gamma-irradiated at 5, 10, 20 and 50 kGy in aqueous solution were evaluated using SDS-PAGE, immunoblotting ELISA, and competitive indirect enzyme-linked immunosorbent assay (Ci-ELISA) with sera from peanut hypersensitive patients. 2) The effect of splenocyte stimulation with gamma-irradiated peanuts in mice that were allergic to peanuts - Mice were sensitized to peanuts by intragastric exposure to non-irradiated PN extracts at day 0, 1, 2, and 7 and then challenged

at day 21. Four weeks later, we evaluated the cytokine production patterns and proliferation responses of splenocytes which had been stimulated with 0 kGy non-irradiated peanuts, 10 kGy irradiated peanuts, and 50 kGy irradiated peanuts. 3) The effect of intragastric administration of gamma-irradiated peanuts in mice – Mice were sensitized with non-irradiated PN extracts and irradiated PN extracts intragastricly at day 1, 2, 3, and 7 and then challenged them at day 21. Mice in group Ⅰ (n=5) received crude PN (0 kGy), whereas mice in group Ⅱ (n=5) received 10 kGy irradiated PN, mice in group Ⅲ (n=5) received 50 kGy irradiated PN, and mice in group Ⅳ (n=5) were the naive control. We then analyzed the PN-specific serum IgE, IgG_1, IgG_{2a} antibody levels, PN-stimulated splenocyte cytokine production patterns and splenocyte proliferation responses.

Results: 1) The physicochemical and imunochemical properties of gamma-irradiated peanuts - The disappearance of the protein band after sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the structure of the peanut proteins changed after radiation treatment, and this change may have occurred as a result of fragmentation and/or aggregation of the proteins. In addition, the use of immunoglobulin E (IgE) from peanut-hypersensitive patients allowed the changes in the allergenicity and antigenicity of irradiated proteins to be observed by PN specific IgE immunoblotting and competitive indirect enzyme-linked immunosorbent assay (Ci-ELISA). The results of these analyses indicated that epitopes on the peanut allergens were structurally altered by gamma irradiation. 2) Effect of splenocyte stimulation with gamma-irradiated peanuts in mice that were allergic to peanuts – Gamma-irradiated PN-stimulated productions of IL-10 and IFN-γ were increased when compared to

the non-irradiated PN-stimulated production. In addition, the Th1 / Th2 ratio increased in response to treatment with gamma-irradiated peanuts. 3) Effect of intragastric administration of gamma-irradiated peanuts in mice – The PN-specific IgE level was decreased in group Ⅲ (50 kGy irradiated peanut treatment) when compared to that of group Ⅰ(0 kGy non-irradiated peanut treatment). However, the PN-specific IgE level was increased at week 4 in group Ⅱ (10 kGy irradiated peanut treatment) when compared to group Ⅰ. Furthermore, the PN stimulated IL-4 and IL-10 levels in the 72 hour splenocyte culture supernatant were decreased in group Ⅲ, whereas the PN-stimulated production of IFN-γ was increased group Ⅱ and Ⅲ.

Conclusion: The binding ability of patients's IgE to irradiated peanut decreased depending on the dose. Also, SDS-PAGE and an immunoblotting assay revealed that fragmentation of the proteins had occurred, which shows that a structural change may reduce the binding capacity for the epitopes in ELISA. In murine experiment, that stimulation of the splenocytes of mice that were allergic to peanuts and mice themselves by gamma-irradiated peanuts was found to increase the Th1 / Th 2 ratio and IL-10 production. Therefore, this study showed the possibility of developing an immunogen capable of treatment and reduction of food allergy by using gamma-radiation technology. Future studies are required to further evaluate immunotherapy using irradiated peanuts in a murine model of peanut allergy.

Key words : Peanut allergy, γ-irradiation, Murine model, Cytokine, Immunotherapy

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I. INTRODUTION

An allergic response is caused by a mediation of IgE antibody production rate against allergens from sources such as house dust mite, food, animal, grass pollen etc. Allergic disorder is the result of an interaction between exposures of a genetically susceptible individual to an allergen and various environmental factors (Broide, 2001; Shicherer, 2002). In particular, food allergy is defined as immune-mediated adverse reaction to food that occurs in genetically predisposed individuals (Sampson, 2004), and according to several European and American authors, food allergies affect up to 2% of the adult population and up to 8% of children (Orotoni et al., 2001). In spite of this, food allergy is an emerging public health problem, especially in developed countries (Besler et al., 2001), and over 160 food materials have been identified as allergenic. However, only 8 of these foods account for more than 90% of all food allergies (FAO, 1995; Ellman et al., 2002), including egg, milk, peanut, soya, tree-nuts, crustaceans, fish, and wheat. One of these foods is the peanut, which is widely used for the preparation of a variety of foods in the United States, and is also relied on as a protein extender in developing countries.

The best strategy to control food hypersensitivity is the complete avoidance of food allergens, however, because many allergenic foods are present within processed foods, 100% avoidance is rarely possible (Burks and Ballmer-Weber, 2006). When the elimination of foods from the diet or protection of food hypersensitivity has failed, medications such as antihistamines and corticosteroids have been used, however, these provide minimal efficacy and often produce unacceptable side effects in a prophylactic approach (Sampson, 2004;

Burks and Ballmer-Weber, 2006).

Because of these complications, recent studies have focused on identification of food allergen, reduction of allergenicity of food allergen, and investigation of therapeutic approaches. Physical processes (heating, high pressure, microparticulation, ultrafilteration, and irradiation), chemical processes (proteolysis, fermentation, and refining by extraction), and biotechnological approaches have been evaluated to determine if they are capable of removing or reducing the allergencity of food allergens, and processes such as producing extensively or partially hydrolyzed versions of hypoallergenic food have also been employed (Poms and Anklam, 2004).

In addition, immunotherapy studies to determine if the immune response in food allergic patients could be down-regulated have been conducted in several countries. However, there is currently no specific therapy for the treatment of food allergy available (Nowak-Wegrzyn, 2006). In addition, injection of food allergen extracts can induce a high rate of adverse systemic reactions (Oppenheimer et al., 1992; Nelson et al., 1997), therefore this type of therapy is not recommended (Burks, 2003). For this reason, effective preventive and therapeutic strategies for the treatment of food allergies are urgently needed. Current approaches for the treatment and inhibition of IgE-mediated food allergies include anti-IgE therapy, traditional Chinese herbal medicine, peptide immunotherapy, mutated protein immunotherapy, allergen DNA immunization, vaccination with immunositmulatroy DNA sequences, and probiotics (Nowak-Wegrzyn, 2006; Pons et al., 2005).

Gamma irradiation has been applied widely for the preservation of food because microbes and enzymes can be inactivated by the application of variable doses of gamma-

irradiation. Food irradiation is permitted to inhibit sprouting, disinfect insects and parasites, delay physiological ripening, and extend shelf life. In the United State, the Food and Drug Administration (FDA) first approved irradiation for use on food products (wheat and wheat flour) in 1963. Since then, it has been approved for use on a wide variety of products, including vegetables, spices, fruits, poultry, refrigerated and frozen uncooked meat and fresh shell eggs. In Korea, the Korea FDA has also approved the use of irradiation for food items such as potatoes, whole egg powder, and dried spices.

Proteins that have been exposed to irradiation present distinct structural modifications as a result of aggregation, fragmentation, and the modification of amino acids, which, in turn, affects the solubility of proteins, their tertiary and secondary structure, and their immungenecity. Several studies have reported that the antigenicity of proteins is modified by gamma rays (Kume and Matsuda, 1995; Lee et al., 2001; Byun et al., 2004), and that the alterations in gamma irradiated proteins affect their non-irradiated antigenicity and IgE-binding ability as a result of conformational modifications. In addition, studies have been conducted to determine if the use of irradiation to reduce or abolish the allergenicity of egg, milk, wheat, and shrimp is feasible (Lee et al., 2002; Lee et al., 2001; Leszczynska et al., 2003; Byun et al., 2000), and the results have shown that modification of allergens by gamma irradiation is a feasible therapeutic and preventive approach for the treatment of allergies

The goal of this study was to develop an immunogen capable of the treatment and reduction of food allergies using radiation technology. The peanut (PN) was used as a major allergen and cobalt-60 was used as a radiation source. Immunochemical analyses and murine

model experiments were performed to evaluate the allergenecity and immugenecity of irradiated peanuts. Briefly, this study was investigated to determine if an allergy vaccine could be generated using allergens that had been modified by gamma irradiation.

II. MATERIALS AND METOHODS

Part I. The physicochemical and immunochemical properties of gamma-irradiated peanuts

A. Peanut-allergic human sera.

Peanut-allergic human sera were obtained from 7 patients. All of the patients showed elevated peanut-specific IgE levels above the level of diagnostic decision point (> 15.00 kU/L; Sampson, 2001), as determined by the Pharmacia CAP System (Pharmacia Diagnostics, Uppsala, Sweden). In addition, non-allergic human sera were obtained from 3 patients, who showed undetectable level of peanut-specific IgE (< 0.35kU/L).

B. Preparation of peanut protein extract

Peanuts were ground in a mortar, and the fat was then extracted from the powdered peanuts using petroleum ether in an extractor in a well-ventilated chemical hood. The ether extracted peanuts were then allowed to dry until no residual ether odor was noticeable, after which the protein was extracted by mixing 1 g of ground peanuts with 20 mL of PBS and then stirring for 16 hr at 4℃. Next, the aqueous fraction was collected by centrifugation (1,000 g at 4℃ for 60 min) and then subsequently centrifuged (1,000 g at 4℃ for 60 min) to remove residual traces of fat and insoluble particles. The bicinchonicni acid (BCA) technique (Pierce Chemical Co., Rockford, IL, USA) was then used for protein determination following the manufacturer's instructions using bovine serum albumin (BSA)

Table. 1. Laboratory profile of peanut hypersensitivity patients

Groups	No.	Sex	Age (month)	Total IgE* (IU/mL)	Peanut-specific IgE * (kU/L)
Peanut-allergic patients †	1	F	6	5000	100
	2	M	8	1000	100
	3	M	8	833	56.9
	4	M	20	600	100
	5	M	23	891	100
	6	F	14	276	36.1
	7	M	10	1193	100
Non-allergic controls	8	F	9	21	<0.35
	9	F	44	61	<0.35
	10	M	27	14	< 0.35

* Total IgE and peanut-IgE were measured by Pharmacia CAP System. † Diagnostic decision point of peanut-specific IgE is above 15.00 kU/L.

as a standard.

C. Gamma-irradiation of peanut protein extract

The peanut protein extract solution (10 mL) was put into a glass tube with a cap and then irradiated using a cobalt-60 irradiator (IR79, Nordion International Ltd., Ontario, Canada) equipped with 100-kCi activity at 10 ± 0.5℃ and operated at a dose rate of 10 kGy h^{-1}. The applied dosage levels were 5, 10, 20, and 50 kGy, and the absorbed dose was monitored using both a free-radical and a ceric/cerou dosimeter (Holm and Berry, 1970). After irradiation, the tubes were stored at 4℃. Non-irradiated solution was used as a control.

D. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis was carried out using precast 4-20% Tris-glycine gels (Novex ®, Invitrogen, San Diego, CA, USA) at 125 V in a SDS running buffer system according to the manufacturer's instructions. A SeeBlue® Plus^2 Pre-stained standard that was purchased from Invitrogen was used to determine the molecular masses of the protein bands. The marker consisted of proteins with the following molecular masses: 98, 64, 50, 36, 22, and 16 kDa. The gel was visualized by staining it with Commassie Brilliant Blue R 250 (Sigma Co., St. Louis. MO, USA) and the gel images were captured using a Gel Doc Image Analysis system (Bio-rad, Hercules, CA, USA). Band quantitation was performed by densitometry using Multi Gauge Version 3.0 (Fuji Photo Film Co., Tokyo, Japan).

E. Immunoblotting

Protein bands were electro transferred from the gel to a nitrocellulose membrane (Millipore Co., Bedford, MA, USA) using a modified version of the method described by Towbin et al. (1979). The membrane was then blocked with 3% bovine serum albumin (BSA) for 4 hr, followed by washing with 0.05% Tween 20-phospate buffered saline (PBS). Peanut-allergic human pooled sera (diluted at 1:10) and control pooled sera (diluted 1:10) were then added to the membrane for >16 hr at 4℃. After being washed, the samples were then conjugated with alkaline phosphatase conjugated goat anti-human IgE (Sigma Co.) diluted 1: 1,000. The membrane was then developed using Sigma *FAST* ™ BCIP/NBT (5-Bromo-4-Chloro-3-indolyl phosphate/Nitro bule tetrazolium, Sigma Co.), followed by a washing step, after which development was terminated using H_2O. The blot images were captured using a Gel Doc Image Analysis system (Bio-rad), and the binding capacity was evaluated by densitometry using Multi Gauge Version 3.0 (Fuji Photo Film Co.).

F. Stability in protease

The protease solutions were prepared as described previously. Briefly, the protease solution was prepared by dissolving 1 mg of trypsin (from bovine pancreas, Sigma Co.) in 1 mM hydrochloric acid and a sufficient volume of water to give a final volume of 10 mL and a final pH of approximately 7.0. Two microgram per milliliter of non-irradiated peanut extract (0 kGy) and irradiated peanut extracts (10, 20 and 50 kGy) were then added to the trypsin solution and digested by continuous shaking for 30 min at 37 ℃. The trypsin-digested samples were then separated by SDS-PAGE on a 4-20% Gradient Tris-glycine Gel

(Invitrogen) at 125 V in a SDS running buffer (Invitrogen) system according to the manufacturer's instructions.

G. Enzyme-linked immunosorbent assay (ELISA)

One microgram per milliliter of non-irradiated (0 kGy) or irradiated peanut extracts (5, 10, 20, and 50 kGy) was coated with 0.1 M bicarbonate coating buffer (pH 9.6) overnight at 4℃. The wells were then washed 3 times with PBS containing 0.05% Tween-20 (PBS-T) and then used in the subsequent steps. After blocking with 10% FBS in PBS for 2 hr, peanut-allergic human pooled sera (1:300) and control pooled sera were added to the wells and incubated for 2 hr. Next, biotinylated anti-human IgE (Vector Laboratories, Burlingame, CA, USA) was added, followed by a washing step. Streptoavidin-horseradish peroxidase conjugate (diluted at 1:1,000; BD PharMigen, San Diego, CA, USA) was then added for 15 min. TMB solution (One 3,3′,5,5′-tetramethylbenzidine, BD PharMigen) was added as a chromogen and the reaction was stopped by adding 0.5 M H_2SO_4 without washing. The absorbance of the plate at 450 nm was then read using a microplate reader (Benchmark. Bio-rad).

H. Inhibition ELISA

One microgram per milliliter of 0 kGy non-irradiated peanut extract was coated with 0.1 M bicarbonate coating buffer (pH 9.6) overnight at 4℃. Next, the wells were washed with PBS containing 0.05% Tween-20 (PBS-T) three times and then used in the subsequent steps. During blocking with 10% FBS in PBS for 2 hr at 37℃, peanut-allergic human pooled sera

(1:300) and inhibitor (0.01 ㎍/mL, 0.1 ㎍/mL, 1 ㎍/mL, 10 ㎍/mL, 100 ㎍/mL) were added to same tube and pre-incubated for 2 hr at 37℃. After the microplate was washed, 100 μL of pre-incubated peanut-allergic human sera and serially diluted inhibitor were added to the blocked wells and then incubated for 2 hr. Next, biotinylated anti-human IgE (Vector Laboratories) was added, which was followed by a washing step. Streptoavidin-horseradish peroxidase (diluted at 1:1,000; BD PharMigen, San Diego, CA, USA) was then added for 15 min. TMB solution (BD PharMigen) was then added as a chromogen and the reaction was stopped by adding 0.5 M H_2SO_4 without a washing step. The absorbance of the plate at 450 nm was then read using a microplate reader.

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

I. Competitive indirect ELSIA (Ci-ELSIA) using IgE and IgG antibodies obtained from the patients.

The ability of IgE and IgG obtained from the patients to bind to the irradiated proteins was tested by Ci-ELSIA. Briefly, polystyrene flat-bottom microtiter plates (Maxisorp, Nunc, Roskide, Denmark) were coated with 100 μL of peanut protein (10 ㎍/mL) in 0.1M sodium bicarbonate buffer (pH 9.6) overnight at 4℃. All subsequent steps were performed at 37℃. The plates were washed three times with PBS containing 0.05% (v / v) Tween 20 (PBS-T). To reduce nonspecific binding, the plates were then blocked by incubation for 2 h with 200 μL of PBS containing 10% (v / v) fetal bovine serum. After washing, 50 μL of different serial

dilutions of the sample solution ranging from 100 to 1 μg/mL in PBS were added to the blocked wells, and then 50 μL of the patient's sera that had been diluted 50 times in PBS was added. Next, the plates were incubated for 2 hr and then washed five times with PBS-T. After 100 μL of 1:1,000 diluted biotinylated human anti-IgE (Vector Laboratories) was added to the wells, the plates were incubated for 1hr. Next, the plates were washed, and then 100 μL of TMB (BD PharMigen) was added to induce a color reaction for 10 min that was then stopped by adding 2 N H_2SO_4 (50 μL /well). The absorbance at 450 nm was then measured using an ELISA reader (Benchmark, Bio-rad). The formation of the Ci-ELSIA to human IgG was conducted following the same method as was used to analyze the human IgE, however, the dilution rate of the human IgG solution was 1:500.

J. Statistical analysis.

Statistical analyses were performed using the Student's *t*-test for two group comparisons and using one-way ANOVA to compare more than two groups. A *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 12.0 for window software (Chicago, IL, USA).

Part II. The effect of splenocyte stimulation with gamma-irradiated peanuts in mice that was allergic to peanut

A. Mice and reagents

Four-week-old female C3H/HeJ mice were purchased from SLC-Japan (Hamamatsu, Japan) and maintained on peanut free chow, under specific pathogen-free conditions. Standard guidelines for the care and use of animals were followed (Guide for the care and use of Laboratory Animals, National Institute of health publication).

Cholera toxin (CT) was purchased from List Biological Laboratories, Inc (Campbell, CA, USA). Concanavalin A (Con A) was purchased from Sigma Co. Antibodies for IgE, IgG_1, and IgG_{2a} measurements by means of ELISA and ELISA Set for cytokine measurements were purchased from BD PharMingen.

B. Intragastric sensitization / challenge

Mice were sensitized intragastrically with 1 mg of 0 kGy non-irradiated peanut extract (G1, n=5) plus cholera toxin as adjuvants on experimental days 0, 1, 2, and 7. Three weeks after this initial sensitization, mice were challenged intragastrically with 0 kGy non-irradiated peanut extract at a dose of 10 mg / mouse divided into 2 doses that were administered 30 min apart. Intragastric feeding was performed using a stainless steel gavage needle, as described previously (Li et al., 2000). Naive mice (G2, n=5) served as control (Fig. 1).

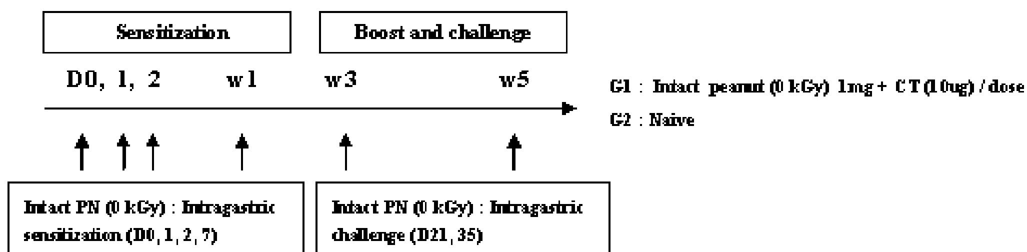


Fig. 1. Protocol for experiment B. Female C3H/HeJ mice (4 week old, 5 per group) were sensitized and challenged via the intragastric route with 1 mg of non-irradiated peanut extract (0 kGy) mixed in cholera toxin (10 μg) as an adjuvant. (PN : peanut, CT : cholera toxin, D: day, W : week).

C. Measurement of peanut-specific IgE, IgG_1 and IgG_{2a}

Tail vein blood was obtained at weekly intervals following the initial sensitization. Sera were collected and stored at -20℃ until the levels of peanut-specific IgE were measured by ELISA. Briefly, Maxisorb Immuno-plates (Nunc) were coated with 2 ㎍/mL of crude peanut extract in coating buffer, pH 9.6 (Sigma). After an overnight incubation at 4℃, the plates were washed and blocked with 10% FBS-PBS for 1 hr at RT. Following 3 washes, serum samples (1:10 diluted) were added to the plates, which were then incubated overnight at 4℃. Next, the plates were washed 3× and 100 μL of biotinylated rat anti-mouse IgE (0.5 ㎍/mL) was added to each well. The plates were then incubated for 1 hr at RT, washed 3×, and 100 μL of avidin peroxidase (BD PharMingen) (1:2,000 dilution) was added for an additional 30min at room temperature. The plates were then washed 6× and TMB (BD PhraMigen) was added for 30 min at RT, after which the reaction was stopped with 2 N H_2SO_4. The absorbance of the wells at 450 nm was then read using a microplate reader (Bio-Rad), and the levels of IgE were then calculated using a standard curve for measuring monoclonal antibody based on the ELISA method for total IgE (BD PharMingen).

To determine the levels of peanut-specific IgG_1 and IgG_{2a}, plates were coated with crude peanut extract and then blocked and washed in the same manner as described above. Serum samples (1:500 dilution) were added to the plates, which were then incubated overnight at 4℃. Next, the plates were washed and biotinylated rat anti-mouse IgG_1 or IgG_{2a} monoclonal antibodies (1 ㎍/mL) were then added to the plates to detect IgG_1 and IgG_{2a}, respectively. The plates were then incubated for an additional 1 h at room temperature. After being washed, avidin peroxidase (BD PharMingen) was added for an additional 15 min at

room temperature, followed by an additional 8 washes. The reactions were then developed with TMB (BD PharMingen) for 15-30 min at room temperature, stopped with 2 N H_2SO_4 and read at 450 nm using a microplate reader. All analyses were performed in duplicate.

D. Proliferation assays

Spleens were removed from peanut-allergic mice and naive mice after re-challenge with 0 kGy non-irradiated peanut extract at week 5. Spleen cells were then isolated and suspended in complete culture medium (RPMI 1640 plus 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% glutamine), until being incubated in triplicate cultures in 96-well plates (1×10^6 / well in 0.2 mL) in the presence or absence of 0 kGy non-irradiated peanut extract, 10 kGy irradiated peanut extract, or 50 kGy irradiated peanut extract (50 ㎍/mL). Cells stimulated with Con A (2 ㎍/mL) were used as a positive control. Two days later, the cultures were pulsed for 18 hr with 1 Ci per well of ^{3}H-thymidine. The cells were then harvested and the incorporated radioactivity was counted in a β-scintillation counter (TRI-Carb 2100R, PACKRD, Meriden, CT, Canada). Results are expressed as ratios of counts per minute (cpm) of un-stimulated- versus stimulated-wells.

E. Quantification of cytokines in splenocyte culture supernatant

Splenocytes were cultured in 24 well plates (4×10^6 /well/mL) in the presence or absence of 0 kGy non-irradiated peanut extract, 10 kGy irradiated peanut extract, or 50 kGy irradiated peanut extract (50 ㎍/mL). Cells stimulated with Con A (2 ㎍/mL) were used as a positive control. IL-4, IL-6, IL-10, IL-13 and IFN-γ levels in supernatants collected after

72 hr of culture were then determined by ELISA according to the manufacturer's instructions. (BD PhraMigen).

F. Statistical analysis

Statistical analyses were conducted using the Student's *t*-test for two group comparisons. A *P* value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 12.0 for windows software (Chicago, IL, USA).

Part III. Effects of intragastric administration of gamma-irradiated peanuts in mice

A. Mice and reagents

The mice and reagents used in this experiment were prepared as described in Part II.

B. Intragastric sensitization / challenge of non-irradiated peanut extract and irradiated peanut extracts

Mice were sensitized and challenged intragastrically with 1 mg of 0 kGy non-irradiated peanut extract (G1, n=5), 10 kGy irradiated peanut extract (G2, n=5), or 50 kGy irradiated peanut extract (G3, n=3) and cholera toxin (10 ㎍/mouse) weekly for 3 weeks, as above described (Fig. 2). Intragastric feeding was performed using a stainless steel gavage needle, as described previously. Naive mice served as a control (G4, n=5).

C. Measurement of body weight during the experiment

To evaluate the effects of intragastircally administered irradiated peanuts, the body weight of each group of mice was measured weekly using a digital balance (Mettler, Columbus, OH, USA).

D. Measurement of peanut-specific IgE, IgG_1 and IgG_{2a}

Peanut-specific IgE, IgG_1 and IgG_{2a} were evaluated using the same method as described in Part II.

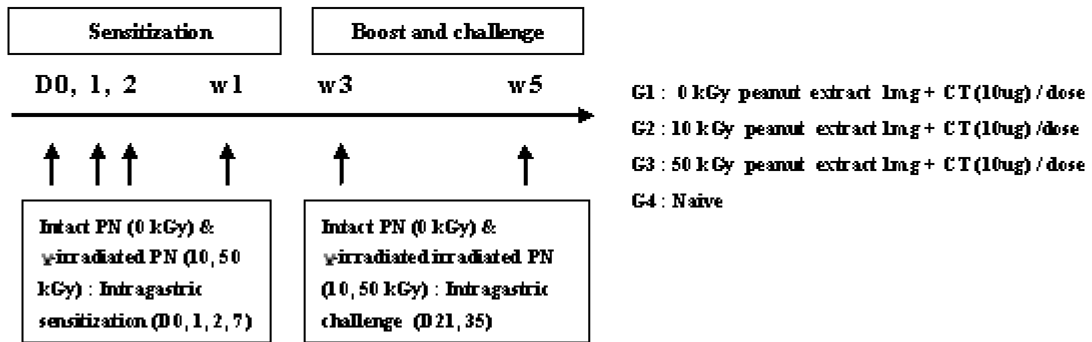


Fig. 2. Protocol for experiment C. Female C3H/HeJ mice (4 week old, 5 per group) were sensitized and challenged via the intragastric route with 1 ㎎ of non-irradiated peanut extract (0 kGy) and γ-irradiated peanut extract (10, 50 kGy) mixed in cholera toxin (10 ㎍) as an adjuvant. (PN: peanut, CT : cholera toxin, D : day, W : week).

E. Proliferation assays

The proliferation assay was evaluated using the method described in part II.

F. Quantification of cytokines in the splenocyte culture supernatant

Cytokines were evaluated using the method described in Part II.

G. Statistical analysis

Statistical analyses were performed using the Student's *t*-test for two group comparisons and using one-way ANOVA to compare more than two groups. A P value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 12.0 for windows software (Chicago, IL, USA).

III. RESULTS

Part I. The physicochemical and immunochemical properties of gamma-irradiated peanuts

A. SDS-PAGE

The electrophoretic pattern of peanuts that were gamma-irradiated with 5, 10, 20, and 50 kGy are shown in Fig. 3. All amounts of irradiation caused a decrease in the intensity of the peanut band. In non-irradiated peanuts, bands corresponding to *Ara h* 1 area (58 kDa), *Ara h* 2 area (21 kDa, 19 kDa), and *Ara h* 3 area (41, 33, 25 kDa) were observed, whereas the major bands were not observed when the 10 kGy and 20 kGy irradiated peanuts were evaluated. In addition, when the 50 kGy irradiated peanut protein was evaluated, a smear phenomenon with fragmentation and aggregation was appeared on the gel. This finding is similar to results that have been observed in studies of other proteins, such as β-lactoglobulin (Lee et al., 2001), bovine and porcine serum plasma (Lee et al., 2003), hen's lysozyme (Stevens et al., 1967) and ovomucoid (Moon and Song, 2001). Table 1 summarizes the relative amount of peanut major allergen band patterns in non-irradiated peanut extract and irradiated peanut extracts. As shown in Table 1, the gamma-irradiated peanut major bands decreased in a dose dependent manner, with no bands being observed in 50 kGy irradiated peanut extract. Conversely, relative amount of 25 kDa protein band increased in 10 kGy irradiated peanut extract. Taken together, these results indicated that irradiated peanut proteins were fragmented/ aggregated by gamma irradiation.

B. IgE Immunoblot

All bands of non-irradiated peanuts were detected in the pooled sera obtained from peanut-hypersensitive patients. However, the bands of gamma-irradiated peanuts disappeared from the pooled sera obtained from peanut-hypersensitive patients in a dose dependent manner. The different reactivity against IgE treated with the non-irradiated peanut extract and irradiated peanut extract was demonstrated by a change in the IgE binding epitopes as a result of irradiation, which was considered to indicate that the epitopes of the gamma-irradiated peanuts were disrupted by irradiation. To determine whether a correlation exist between IgE-binding capacity and radiation dose, the relative percent amount of the IgE-binding patterns to peanut major allergen is listed in Table 2. As shown in Table 2, the IgE-binding capacity decreased with gamma-irradiated peanuts in a dose dependent manner. However, IgE-bind capacity of 41 kDa in irradiated peanut extracts increased than that of non-irradiated peanut extract, which indicates that the peanut-specific IgE binding epitopes reform newly in irradiated peanut extract. Overall, a comparison of IgE-binding capacity between non-irradiation peanut and irradiated peanut indicated a proportion correlation between IgE-binding capacity and radiation dose.

C. Stability in protease

To evaluate the stability of the irradiated peanuts in response to protease, non-irradiated peanuts and irradiated peanuts (10, 20 and 50 kGy) were subjected to trypsin digestion. Fig. 5 shows the results of the protease experiments, which indicate that the extent of degradation or loss of gamma-irradiated peanuts after incubation in protease solution was dependent on

the radiation dose. Taken together, these results indicated that peanut proteins were fragmented by gamma irradiation.

D. ELISA

We determined whether irradiated peanuts protein extracts reduced the binding capacity of IgE obtained from peanut-hypersensitive patients by monitoring serum peanut-specific IgE ELSIA. As shown in Fig. 6, the O.D. of the non-irradiated peanut ELISA was 1.45 ± 0.01, and the O.D. of the 5 kGy irradiated peanut ELISA was similar to that of the non-irradiated peanut (1.06 ± 0.03). Conversely, the O.D. of all peanuts subjected to an irradiation dose greater than 10 kGy was significantly lower than that of non-irradiated peanuts (10kGy irradiated peanut 0.52 ± 0.03, 20 kGy irradiated peanut 0.53 ± 0.03, 50 kGy irradiated peanut 0.49 ± 0.04, $P < 0.05$).

E. Inhibition ELISA

ELISA inhibition showed gamma-irradiation dose-dependent inhibition of IgE in sera collected from patients that were positive to peanut (Fig. 7). When 0.1 ㎍ of non-irradiated peanut extract was used as an inhibitor, the inhibition of IgE binding was found to be 75 %, whereas when 0.1 ㎍ of 10 kGy irradiated peanuts were used as an inhibitor, the inhibition of IgE binding was found to be 60 %, and when 0.1 ㎍ of 20 kGy irradiated peanuts were used, the inhibition of IgE binding was found to be 28 %. In addition, when 0.1㎍ of 50 kGy irradiated peanuts was used as an inhibitor, the inhibition of IgE binding was found to be 29 %.

F. Ci-ELISA

The binding ability of IgE of peanu-allergic patient's IgE to irradiated peanuts was determined by Ci-ELISA. Peanut-specific IgE obtained from allergic patients had difficulty recognizing the irradiated peanuts, depending on the dose of irradiation, and the slope of the curve obtained by competition between coated antigen and irradiated protein indicated that conformational changes may occur in the epitopes following irradiation. Specifically, an increase in slope indicates that the ability of the antibody to bind to irradiated protein is reduced. However, the slopes generated as a result of treatment with irradiated peanuts decreased, which indicates that the binding capacity between irradiated peanuts and their antibodies increased. Furthermore, this binding ability was reduced as the radiation dose increased.

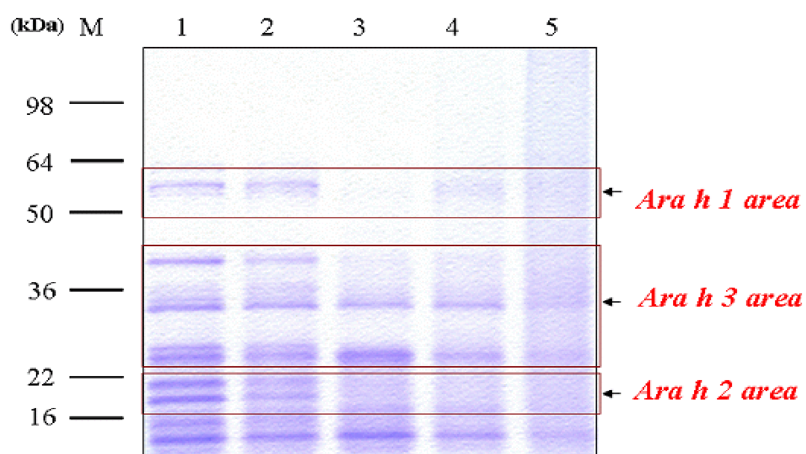


Fig. 3. SDS-PAGE of non-irradiated and irradiated peanuts. (lane 1: 0 kGy non-irradiated peanut, lane 2: 5 kGy irradiated peanut, lane 3: 10 kGy irradiated peanut, lane 4: 20 kGy irradiated peanut, lane 5: 50 kGy irradiated peanut, M: Marker, kDa: kilodalton, *Ara h* 1, 2, 3: peanut major allergens).

Table. 2. Relative amount of peanut major allergen band patterns using densitometry of Multi Gauge Version 3.0.

Allergen area	Molecular Mass (kDa)	0 kGy	5 kGy	10 kGy	20 kGy	50 kGy
Ara h 1 † area	58	100	96	14	65	-*
Ara h 3 area	41	100	64	27	33	-
	33	100	89	77	75	62
	25	100	81	106	69	59
Ara h 2 area	21	100	68	51	39	-
	19	100	67	49	40	-

* Not detected, † Ara h 1, 2, 3 : peanut major allergen

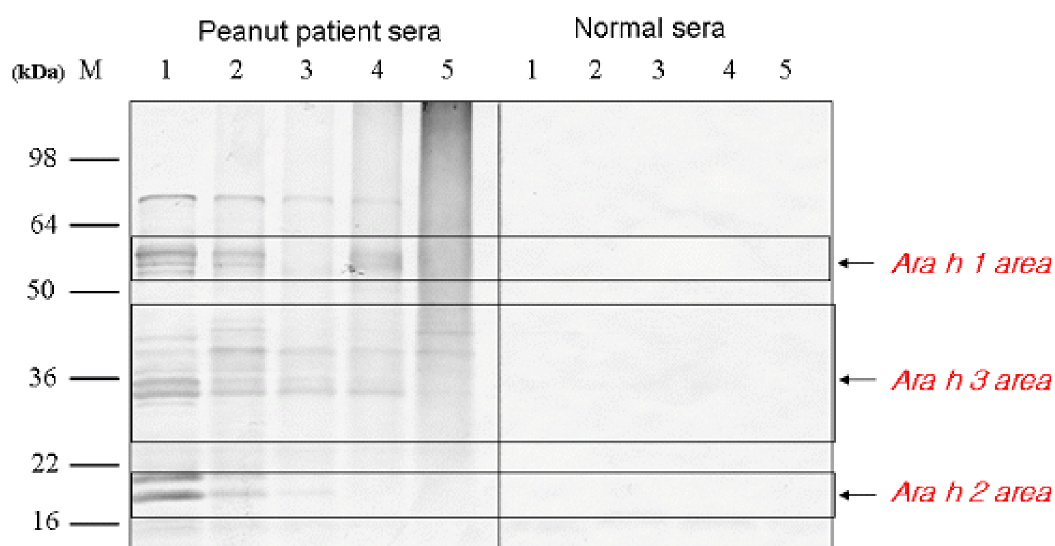


Fig. 4. Peanut-IgE immunoblot of peanut-allergic patient sera and non-allergic control to non-irradiated peanut and irradiated peanuts. (lane 1: 0 kGy non-irradiated peanut, lane 2 : 5 kGy irradiated peanut, lane 3: 10 kGy irradiated peanut, lane 4; 20 kGy irradiated peanut, lane 5: 50 kGy irradiated peanut, M: Marker, kDa: kilodalton)

Table. 3. Relative amount of IgE-binding patterns to peanut major allergen using densitometry of Multi Gauge Version 3.0.

Allergen area	Molecular Mass (kDa)	0 kGy	5 kGy	10 kGy	20 kGy	50 kGy
Ara h 1† area	58	100	57	17	59	-*
Ara h 3 area	41	100	197	125	125	-
	33	100	63	51	55	-
Ara h 2 area	21	100	38	15	12	-
	19	100	35	11	7	-

* Not detected, † Ara h 1, 2, 3 : peanut major allergen

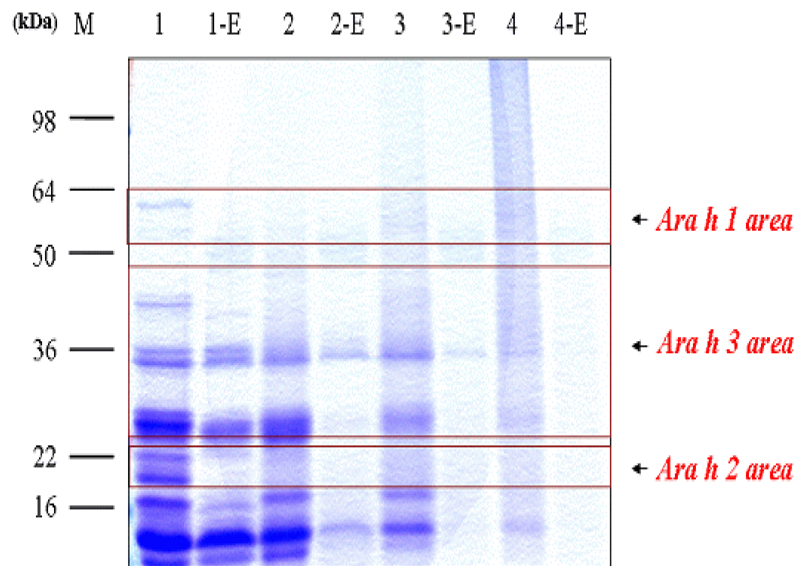


Fig. 5. SDS-PAGE analysis of the protease digestion of non-irradiated and irradiated peanuts. (lane 1: 0 kGy non-irradiated peanut, lane 1-E: enzyme-digested non-irradiated peanut, lane 2: 10 kGy irradiated peanut, 2-E: enzyme-digested 10 kGy irradiated peanut, lane 3; 20 kGray irradiated peanut, 3-E: enzyme-digested 20 kGy irradiated peanut, lane 4: 50 kGray irradiated peanut, 4-E: enzyme-digested 50 kGy irradiated peanut, M: Marker,; kDa: kilodalton; *Ara h* 1, 2: peanut major allergens).

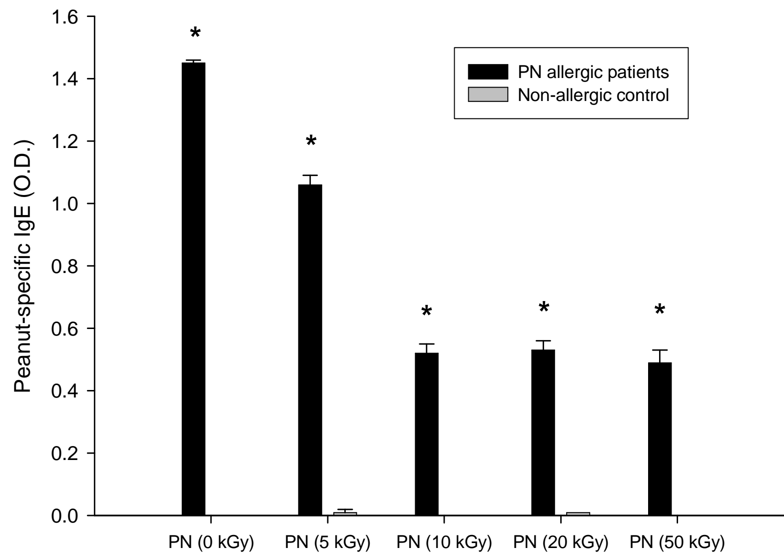


Fig. 6. Comparison of serum specific IgE antibody levels to non-irradiated and irradiated peanuts in pooled sera collected from peanut allergic patients (n=7) and non-allergic controls (n=3). IgE antibodies were measured using ELISA and the relative concentrations were represented by O.D. * $P < 0.05$ by ANOVA.

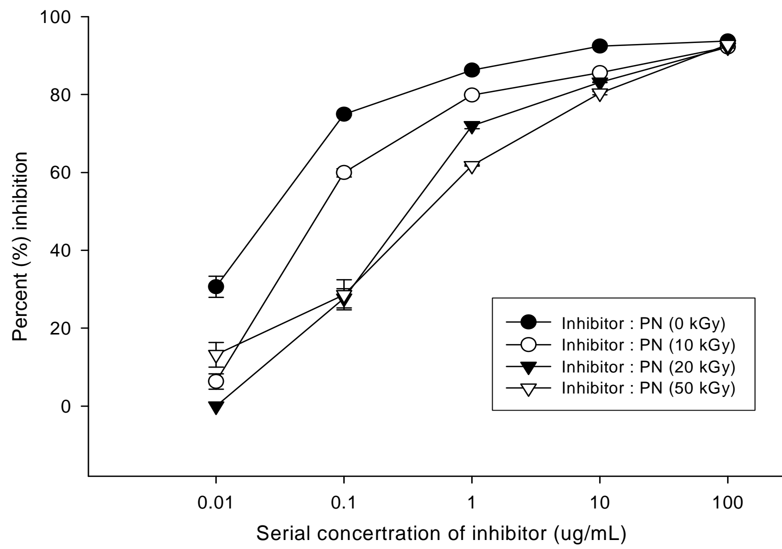


Fig. 7. Comparison of the degree of inhibition of peanut specific IgE ELISA with inhibitors. Serial concentration of inhibitors and pooled sera obtained from peanut allergic patients (n=7) were used.

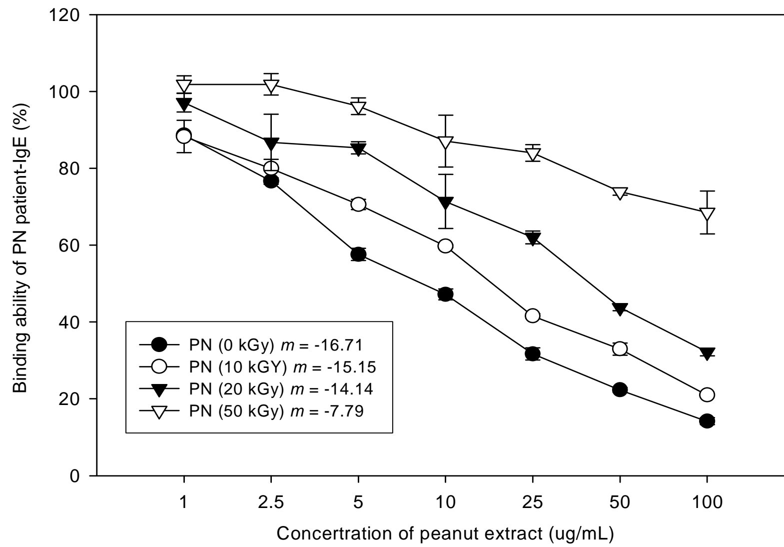


Fig. 8. Binding ability (allergenicity) of peanut allergic patients IgE to protein from irradiated peanuts as determined by Ci-ELISA. The protein solutions were irradiated, serially diluted, and then tested. *m* is the slope of the curve obtained by measuring the binding rate of IgE to coated protein.

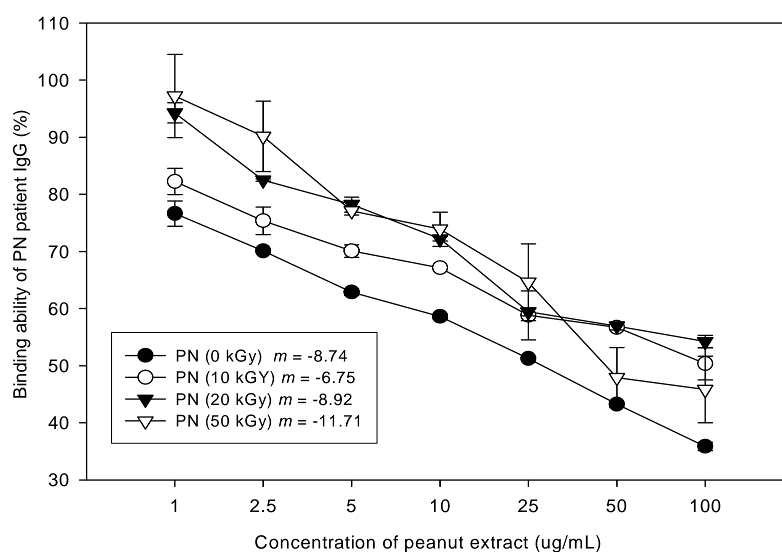


Fig. 9. Binding ability (allergenicity) of peanut allergic patients IgG to protein from irradiated peanuts as determined by Ci-ELISA. The protein solutions were irradiated, serially diluted, and then tested. *m* is the slope of the curve obtained by measuring the binding rate of IgG to coated protein.

Part II. Effects of splenocyte stimulation with gamma-irradiated peanut in mice that were allergic to peanuts

A. Peanut-specific IgE, IgG_1 and IgG_{2a}

To evaluate the peanut-specific IgE response, sera from mice in group 1 were obtained weekly after initial sensitization. The peanut-specific IgE concentration was increased significantly from week 2 through week 4 in group 1. Conversely, there were no significant increases observed in the IgE levels of sera obtained from naïve groups (G2). Furthermore, the peanut-specific IgG_1 and IgG_{2a} concentration was increased significantly from week 3 through week 4 in group 1, however, no significant increases were observed in the IgG_1 and IgG_{2a} levels of the naïve groups (G2). Taken together, these results indicate that the IgE response in group 1 was well developed in peanut-allergic mice.

B. T-cell proliferation Assay

Splenocytes obtained from mice that were challenged with crude peanut extracts were re-stimulated with 0 kGy non-irradiated peanut extract, 10 kGy irradiated peanut extract, and 50 kGy irradiated peanut extract. The level of stimulation induced by peanuts that was irradiated with 10 kGy was similar to the levels induced by stimulation with 0 kGy non-irradiated peanut extract (P =0.286) but those of 50 kGy irradiated peanut extract was significantly decreased (P = 0.010).

C. Cytokine

To determine the levels of cytokines in spleen obtained from mice that were allergic to peanuts, spleen cells were stimulated with 0 kGy non-irradiated peanut extract, 10 kGy irradiated peanut extract, and 50 kGy irradiated peanut extract and then cultured for 72 hr. The IL-4 concentration in splenocyte supernatant obtained from cultures that were stimulated with non-irradiated peanut extract was 30.10±0.00 pg/mL (Fig. 13), which was not significantly different from the IL-4 concentration of splenocyte cultures that had been treated with irradiated peanut extract. In addition, the IFN-γ concentration in the splenocyte supernatant of cultures that had been treated with non-irradiated peanut extract was 1077.98±162.52 pg/mL (Fig. 14), whereas those of the cultures that had been treated with 10 kGy and 50 kGy irradiated peanut extract were 8913±326.18 pg/mL and 2773.63±56.04 pg/mL, respectively, which was significantly different from that of the control (P = 0.008 and P = 0.040). Furthermore, the IL-10 concentration of the splenocyte supernatant obtained from cultures that were treated with 0 kGy non-irradiated peanut extract was 324.73±10.26 pg/mL, which was not significantly different from the concentrations of the splenocytes that were treated with irradiated peanut extract (Fig. 15). In addition, the IL-13 concentration of the splenocyte supernatant obtained from cultures that were treated with non-irradiated peanut extract was 125.40±35.02 pg/mL, which was not significantly different from the concentrations of the supernatants obtained from splenocyte cultures that had been subjected to irradiated peanut stimulation (Fig. 16). Additionally, the IL-6 concentration of the splenocyte supernatant obtained from cultures that had been treated with non-irradiated peanut extract was 178.24±4.95 pg/mL (Fig. 17), whereas the IL-6 concentrations of splenocyte cultures that had been treated with 10 kGy and 50 kGy irradiated peanut extract

were 510.58±5.49 pg/mL and 55.56±3.52 pg/mL, respectively (*P* =0.026 and *P* =0.225). In addition, the Th1/Th2 ratio of the spelnocyte culture supernatant was greater in cultures that were subjected to irradiated peanut stimulation than in those that were subjected to non-irradiated peanut stimulation (Table. 4). These results demonstrated that in vitro stimulation in mice were allergic to peanut with gamma-irradiated peanut protein causes Th 1-dominant peanut-specific responses.

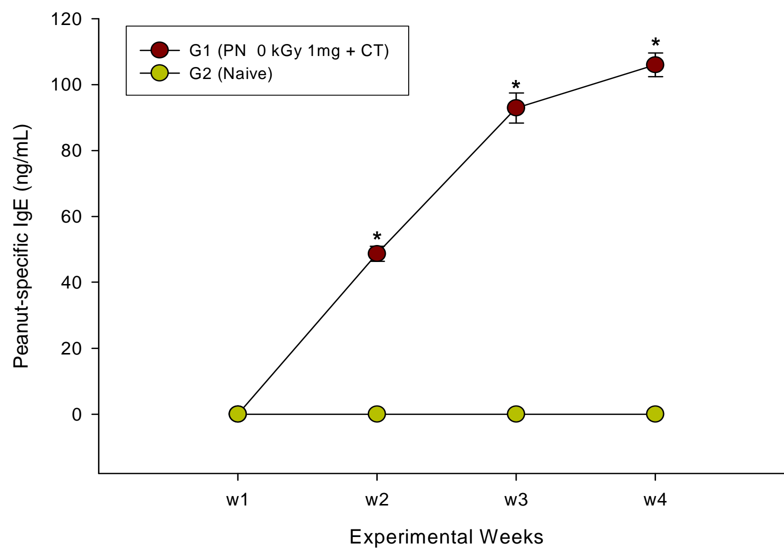
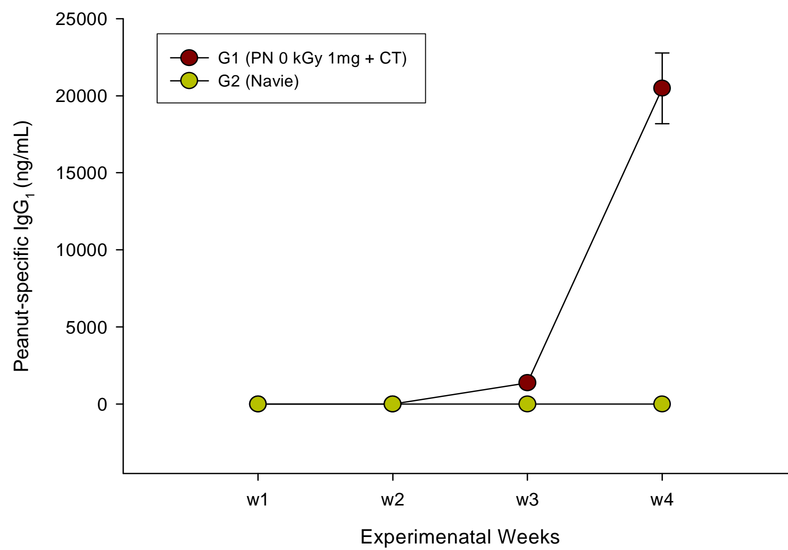


Fig. 10. Levels of peanut-specific IgE in murine model of peanut allergy. Individual sera from different groups of mice (n=5 in each group) were obtained weekly following initial peanut-sensitization. IgE levels were determined by ELISA. Data are given as the mean ± the SEM. * $P < 0.05$ vs. Naive by Student *t*-test.



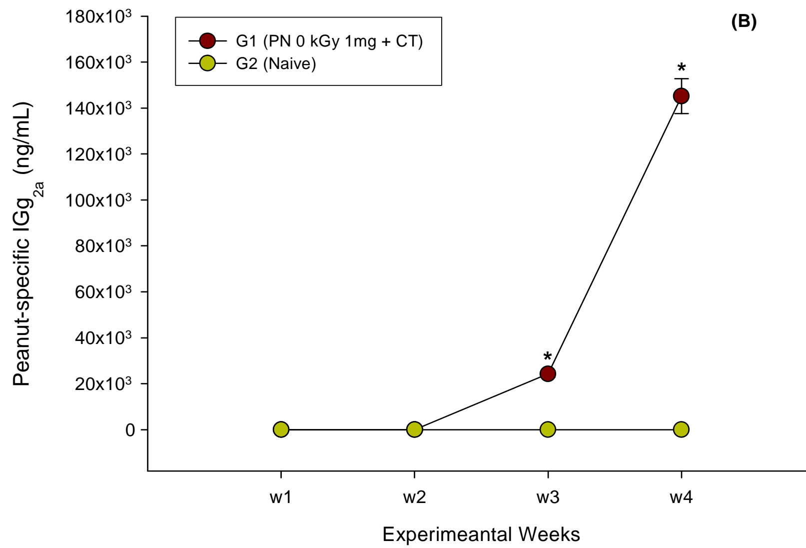


Fig. 11. Levels of peanut-specific IgG_1 (A) and IgG_{2a} (B) in murine model of peanut allergy. Individual sera from different groups of mice (n=5 in each group) were obtained weekly following initial peanut-sensitization. IgG_1 and IgG_{2a} levels were determined by ELISA. Data are given as the mean ± the SEM. * $P < 0.05$ vs. Naive by Student *t*-test.

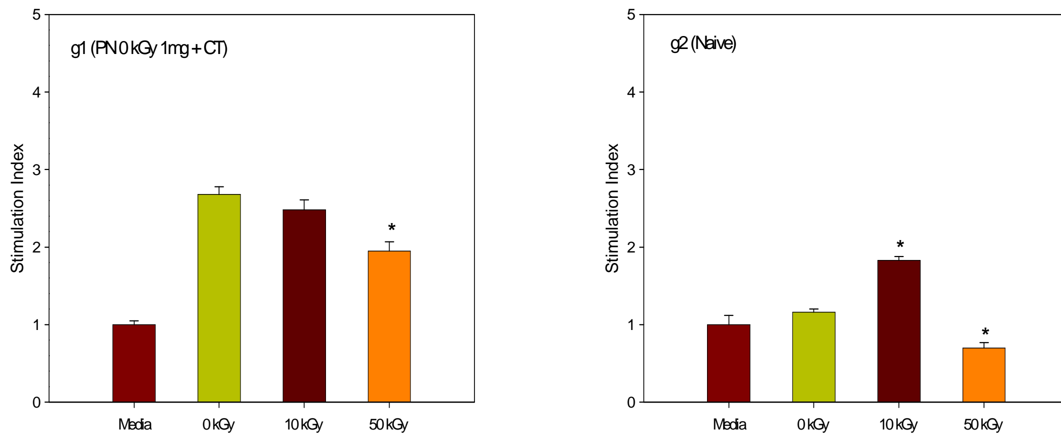


Fig. 12. Proliferative response of splenocytes in response to stimulation with non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut, 10 kGy irradiated peanut, 50 kGy irradiated peanut or media alone. Cells that were cultured in medium only were used as a negative control. Two days later, the cultures were treated with 1 μCi per well of ^{3}H-thymidine, and then incubated for 18 hours, after which the cells were harvested and the incorporated radioactivity was measured. The proliferative potency was given as a stimulation index (SI) over the negative control. * $P < 0.05$: compared with 0 kGy non-irradiated peanut stimulation (by Student t-test).

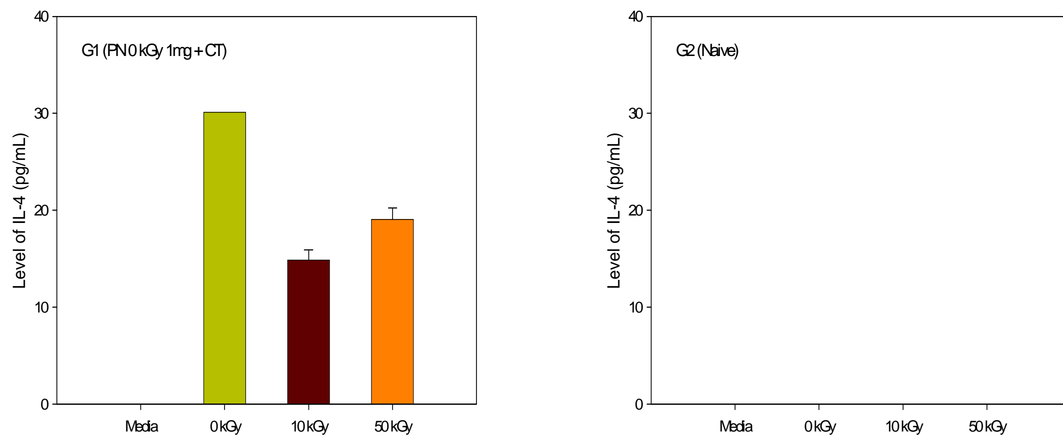


Fig. 13. Level of IL-4 in response to stimulation with non-irradiated peanut extract and irradiated peanut extracts Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut, 10 kGy irradiated peanut, 50 kGy irradiated peanut or medium alone. The cells cultured in medium only served as a negative control. Cytokine levels were determined by analyzing 3-day cultured supernatant by ELISA. Data are given as the mean ± the SEM.

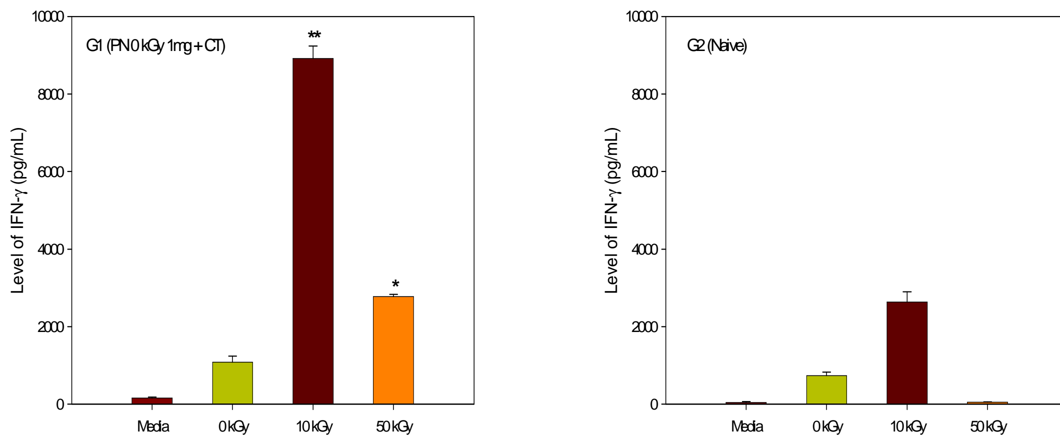


Fig. 14. Level of IFN-γ in response to stimulation with non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut, 10 kGy irradiated peanut, 50 kGy irradiated peanut or media alone. Cells cultured in medium alone served as a negative control. Cytokine levels were determined by analyzing 3-day cultured supernatant by ELISA. Data are given as the mean ± the SEM. * $P < 0.05$, ** $P < 0.01$: compared with 0 kGy non-irradiated peanut stimulation (by Student t-test).

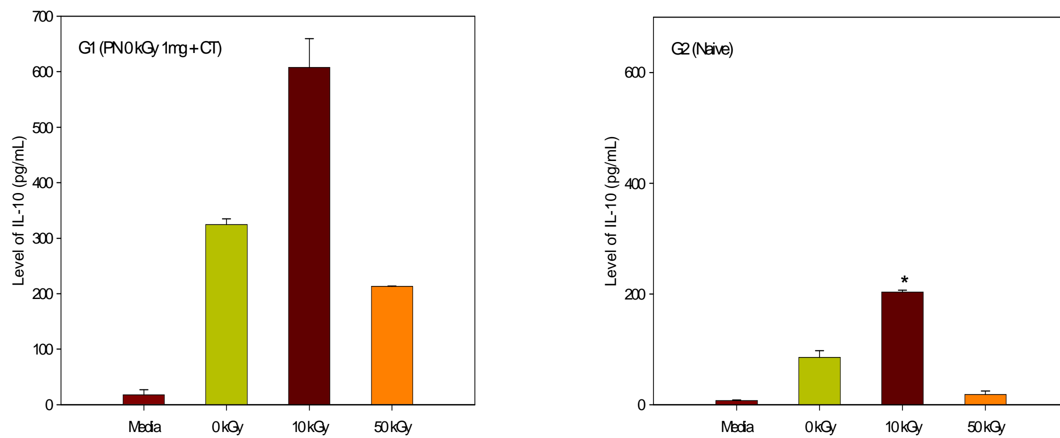


Fig. 15. Level of IL-10 in response to stimulation with non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut, 10 kGy irradiated peanut, 50 kGy irradiated peanut or media alone. Cells cultured in medium only served as a negative control. Cytokine levels were determined by analyzing 3-day cultured supernatant by ELISA. Data are given as the mean ± the SEM. * $P < 0.05$: compared with 0 kGy non-irradiated peanut stimulation (by Student *t*-test).

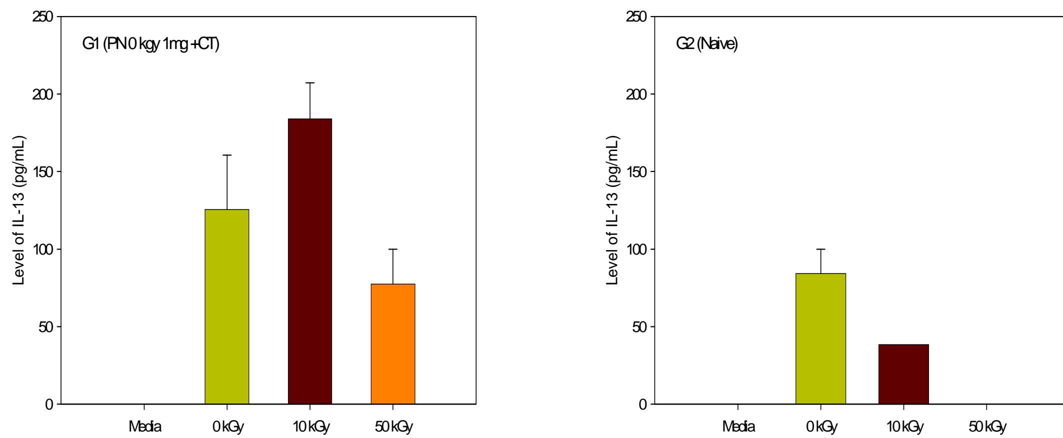


Fig. 16. Level of IL-13 in response to stimulation with non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 ㎍/mL of 0 kGy non-irradiated peanut, 10 kGy irradiated peanut, 50 kGy irradiated peanut or media alone. Cells cultured in medium only served as a negative control. Cytokine levels were determined by analyzing 3-day cultured supernatant by ELISA. Data are given as the mean ± the SEM.

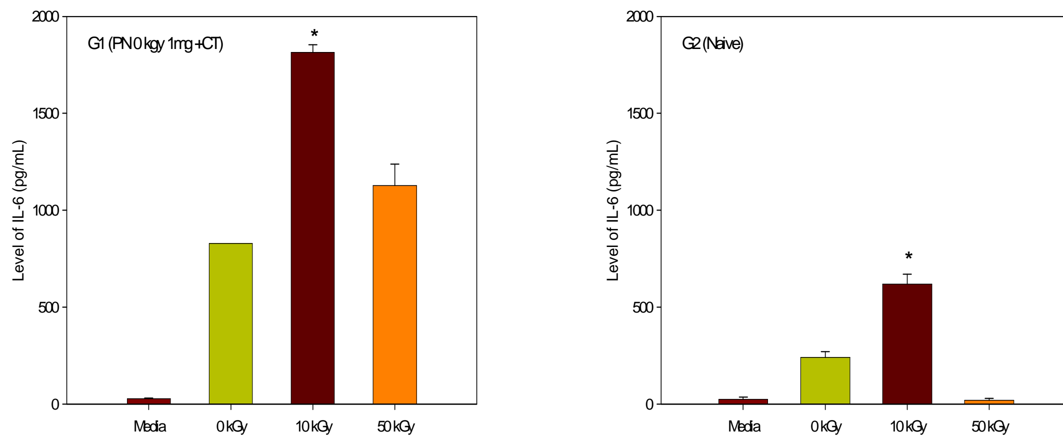


Fig. 17. Level of IL-6 in response to stimulation with non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut, 10 kGy irradiated peanut, 50 kGy irradiated peanut or media alone. Cells cultured in medium only served as a negative control. Cytokine levels were determined by analyzing 3-day cultured supernatant by ELISA. Data are given as the mean ± the SEM. * $P < 0.05$: compared with 0 kGy non-irradiated peanut stimulation (by Student *t*-test).

Table. 4. The levels of IFN-γ, IL-4, IL-6, IL-13, IL-10 and the ratios of non-irradiated peanut & irradiated peanuts stimulated splenocytes cultures.

	Stimulator	IFN-γ (pg/mL)	IL-4 (pg/mL)	IL-6 (pg/mL)	IL-13 (pg/mL)	IL-10 (pg/mL)	IFN-γ/IL-4 (ratio)	IFN-γ/IL-6 (ratio)	IFN-γ/IL-13 (ratio)	IFN-γ/IL-10 (ratio)
G1 (PN 0 kGy 1mg+ CT)	PN0 kGy	1077.98	30.10	828.30	125.40	324.72	35.81	1.30	8.60	3.32
	PN10 kGy	8913.36	14.85	1814.24	183.76	607.52	600.23	4.91	48.51	14.67
	PN50 kGy	2773.63	19.03	1127.11	77.46	213.32	145.75	2.46	35.80	13.00
G2 (Naive)	PN0 kGy	739.71	0	241.11	84.16	85.45	-	3.07	8.80	8.66
	PN10 kGy	2629.39	0	619.81	38.34	203.43	-	4.24	68.58	12.93
	PN50 kGy	53.62	0	19.17	0	18.41	-	2.79	-	2.91

Part III. Effects of intragastric administration of gamma-irradiated peanuts in mice

A. Effect of irradiated peanuts administration on body weight

Mean body weight did not show a significant difference (Table 5), which indicates that the gamma-irradiated peanuts had no effect on body weight.

B. Peanut-specific IgE, IgG_1 and IgG_{2a}

We determined whether protein from the extract of irradiated peanuts reduced peanut-specific IgE by monitoring the serum peanut-specific IgE levels following sensitization, and at the time of challenge. As shown in Fig. 18, 4 weeks after the initial sensitization, peanut-specific IgE levels were elevated in the non-irradiated peanut administration group (G1) and the 10 kGy irradiated peanut administration group (G2). These results indicate that the epitopes reform newly in the 10 kGy irradiated peanut extract. Conversely, the peanut-specific IgE levels were significantly lower in the 50 kGy irradiated administration group (G3). No significant increases in IgG_1 and IgG_{2a} levels (Fig. 19) were observed in the gamma-irradiated groups (G2 and G3).

C. T cell proliferative assay

Splenocytes that were challenged with non-irradiated and irradiated peanuts in each group of mice were re-stimulated with 50 μg/mL of 0 kGy non-irradiated peanut extract. The proliferating activity of splenocyte T cells was found to vary with the dose of radiation (Fig. 20), with the activity of the group that was administered 10 kGy irradiated peanut

extract (G2) being similar to that of group 1 (P = 0.127). Conversely, the activity of the group that was administered extract from peanuts that were irradiated with 50 kGy (G3) was increased than that of group 1, which was not significant (P = 0.066).

D. Cytokines

To determine the role of T cells and cytokines in response to the administration of non-irradiated and irradiated peanuts in mice, the production of cytokines were examined by spleen cells obtained from each group in vitro after they were stimulated with non-irradiated peanut extract and cultured for 72 hr. The IL-4 concentration in splenocyte supernatant taken from the group that was administered extract from 0 kGy non-irradiated peanuts (G1) was 30.10±0.00 pg/mL (Fig. 21), which was not significantly different from the levels of the irradiated peanut administration groups (G2 and G3). In addition, the IFN-γ concentration in the splenocyte supernatant of the group that was treated with non-irradiated peanut extract (G1) was 1077.98±162.52 pg/mL (Fig. 22), whereas those of the groups that were treated with group 2 and group 3 were 3352.86±13.72 pg/mL and 2652.18±188.20 pg/mL, respectively, which was significantly different from that of the group 1 (P = 0.044 and P = 0.025). Additionally, the IL-10 concentration of the splenocyte supernatant of the group 1 was 324.73±10.26 pg/mL, which was not significantly different from that of group 2, althoμgh it was significantly different from that of group 3, Furthermore, the IL-10 levels in the group3, were lower than those of group 1. Moreover, the IL-13 concentration of the splenocyte supernatant of the group G1 was 125.40±35.02 pg/mL, which was not significantly different from those of group 2 and group 3. Additionally, the IL-6

concentration of the splenocyte supernatant of group 1 was 828.30±0.00 pg/mL (Fig. 23), whereas the IL-6 levels of group 2 and group 3 were 1707.18±21.55 pg/mL and 541.65±1.52 pg/mL, respectively, which was significantly different from that of group 1 ($P = 0.016$ and $P = 0.003$). In addition, the Th1 / Th2 ratio was greater in groups 2 and group 3 than group 1 (Table 6). These results demonstrated that intragastric administration in mice with gamma-irradiated peanut protein causes Th 1-dominant peanut-specific responses.

Table 5. Body weight of each group of mice in experiment B

Groups	w1 (g)	w2 (g)	w3 (g)	w4 (g)
G1 (PN 0 kGy 1mg + CT)	17.20±0.58*	19.00±0.45	20.00±0.45	20.80±0.66
G2 (PN 10 kGy 1mg + CT)	16.40±0.51	17.80±0.58	19.60±0.51	20.80±1.02
G3 (PN 50 kGy 1mg + CT)	17.50±0.50	19.00±0.00	20.00±0.00	21.00±0.00
G4 (Naive)	19.83±0.48	20.83±0.70	21.17±0.75	22.63±0.67

* This value was presented as the mean ± the SE.

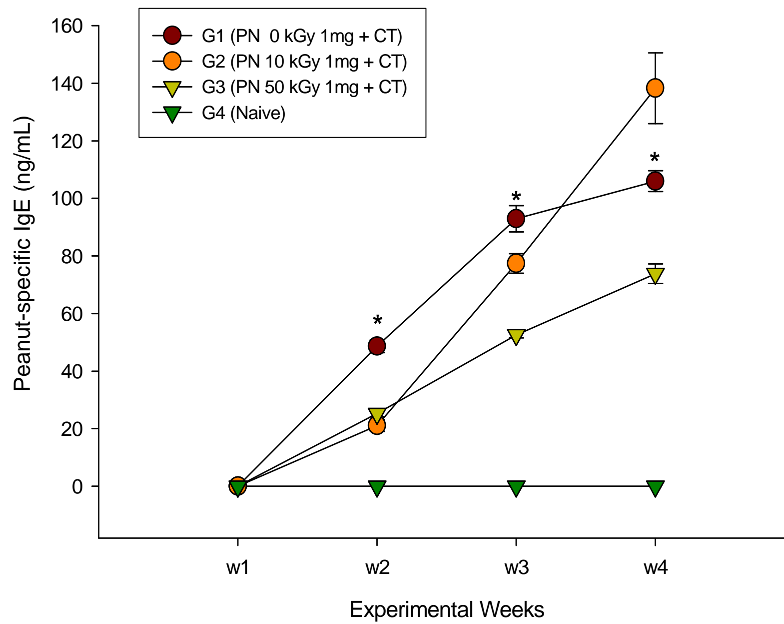


Fig. 18. Level of peanut-specific IgE in mice administrated non-irradiated peanut extract and irradiated peanut extracts. Individual sera from different groups of mice (n=5 in each group) were obtained weekly following initial peanut-sensitization. IgE levels were determined by ELISA. Data are given as the mean ± the SEM. * $P < 0.05$ by ANOVA among G1, G2 and G3.

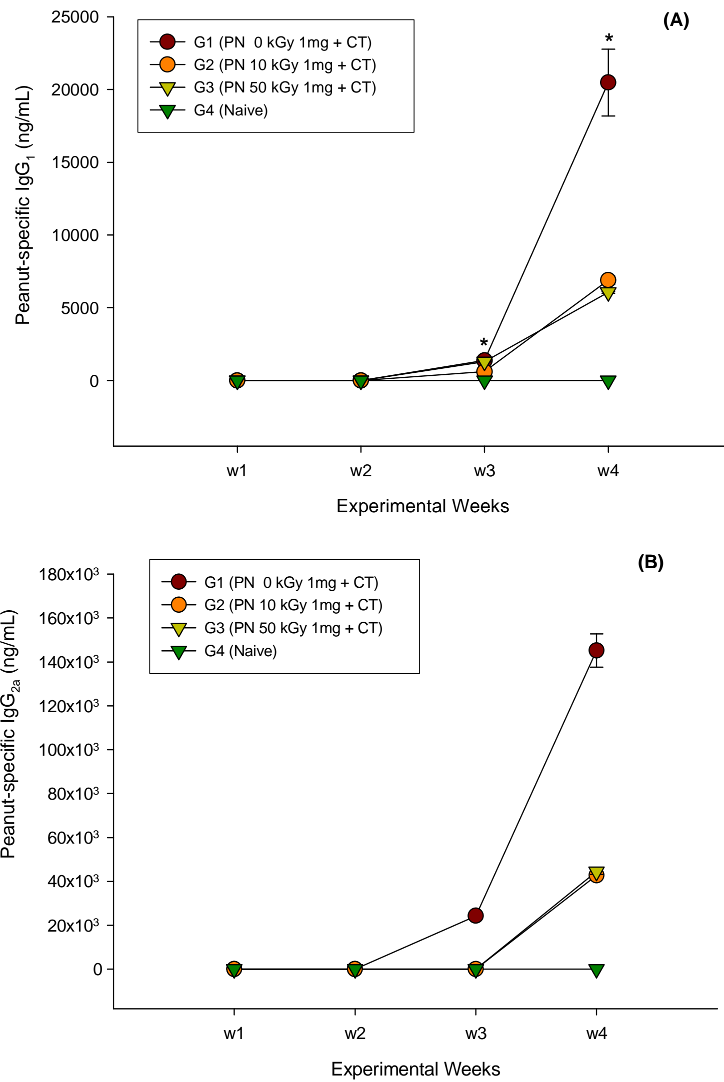


Fig. 19. Levels of peanut-specific IgG_1 (A) and IgG_{2a} (B) in mice administrated non-irradiated peanut extract and irradiated peanut extracts. Individual sera from different groups of mice (n=5 in each group) were obtained weekly following initial peanut-sensitization. IgG levels were determined by ELISA. Data are given as the mean ± the SEM. * $P < 0.05$ by ANOVA among G1, G2, and G3.

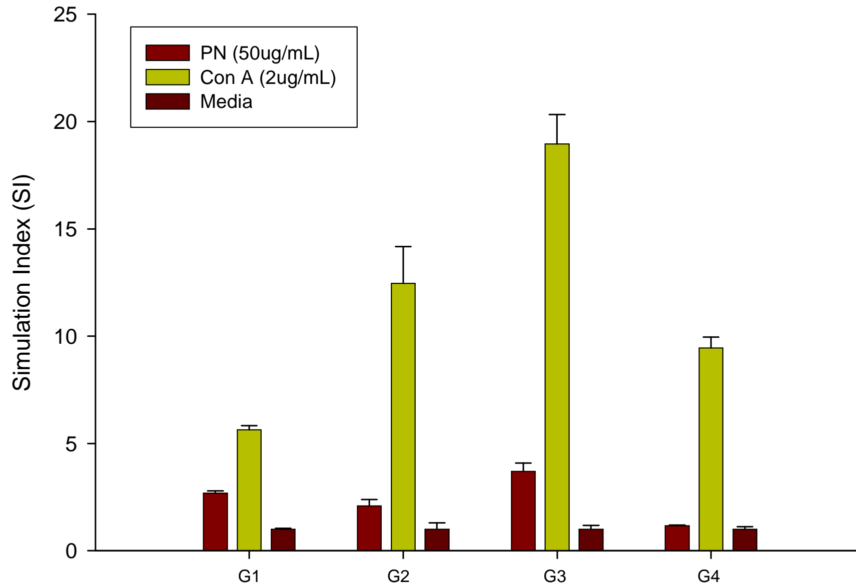


Fig. 20. Proliferative response of splenocytes in response to stimulation with antigen. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of non-irradiated peanut extract or Con A. Cells that were cultured in medium alone served as a negative control. Two days later, the cultures were treated with 1 μCi per well of ^{3}H-thymidine. Cells were harvested 18 hours after treatment and the incorporated radioactivity was then counted. The proliferative potency was given by the stimulation index (SI) over negative control.

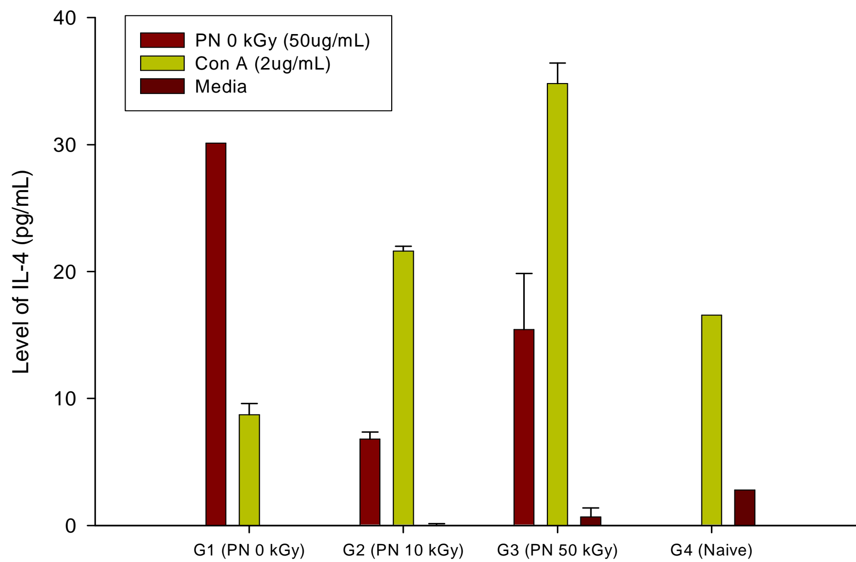


Fig. 21. Level of IL-4 in mice administrated non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut extracts or Con A (2 μg/mL). Cells that were cultured in medium only served as a negative control. Cytokine levels were determined by analyzing 3-day culture supernatant by ELISA. Data are given as the mean ± the SEM.

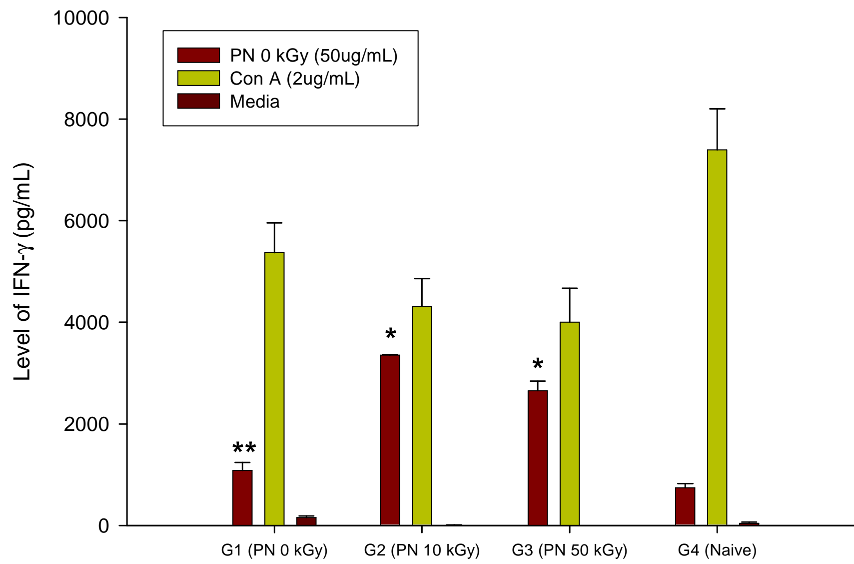


Fig. 22. Level of IFN-γ in mice administrated non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut extracts or Con A (2 μg/mL). Cells that were cultured in medium alone served as a negative control. Cytokine levels were determined by analyzing 3-day culture supernatant by ELISA. Data are given as the mean ± the SEM. * $P <$ 0.05 vs. ** by Student *t*-test.

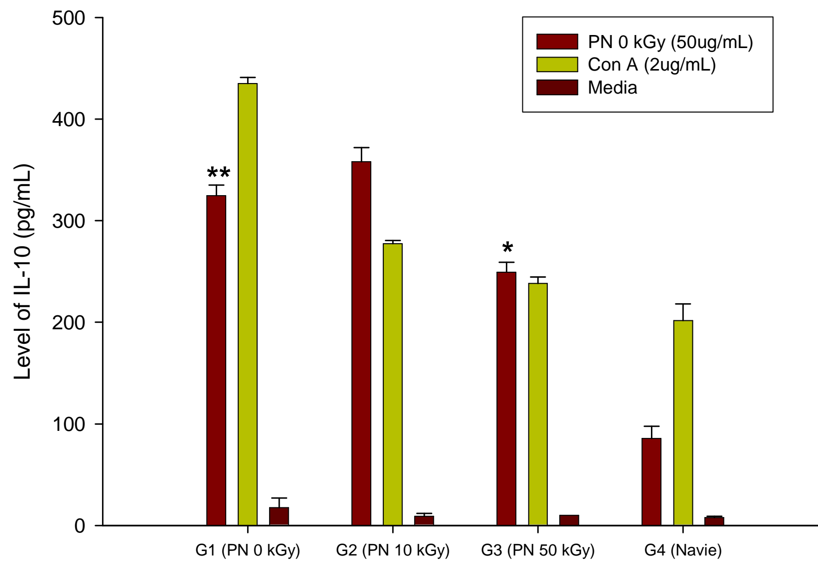


Fig. 23. Level of IL-10 in mice administrated non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut extracts or Con A (2 μg/mL). Cells that were cultured in medium only served as a negative control. Cytokine levels were determined by analyzing 3-day culture supernatant by ELISA. Data are given as the mean ± the SEM. * P < 0.05 vs. ** by Student t-test.

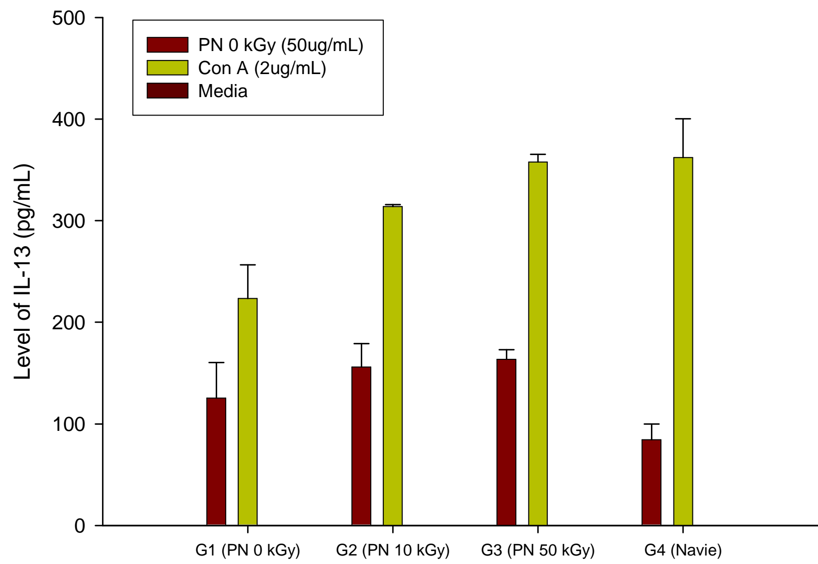


Fig. 24. Level of IL-13 in mice administrated non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut extracts or Con A (2μg/mL). Cells that were cultured in medium alone served as a negative control. Cytokine levels were determined by analyzing 3-day culture supernatant by ELISA. Data are given as the mean ± the SEM.

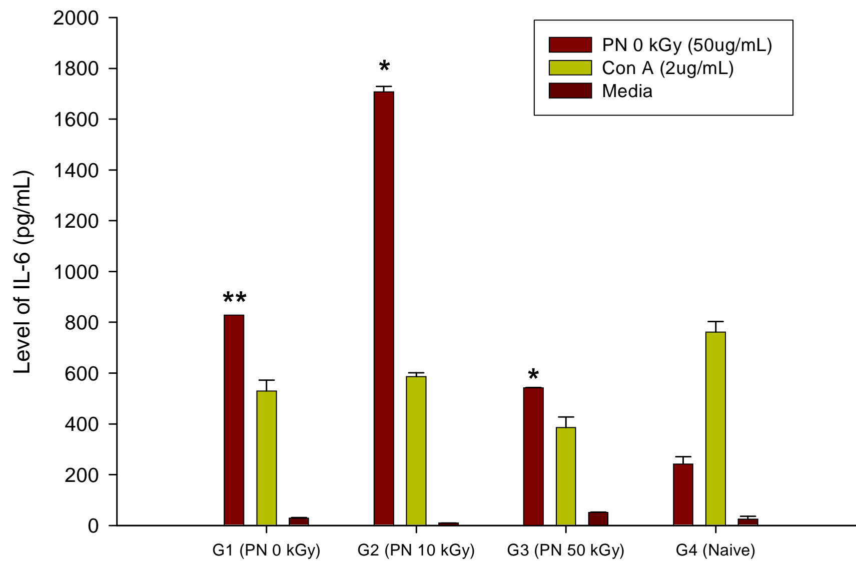


Fig. 25. Level of IL-6 in mice administrated non-irradiated peanut extract and irradiated peanut extracts. Spleen cells from each group of mice (n=2) were stimulated with 50 μg/mL of 0 kGy non-irradiated peanut extracts or Con A (2 μg/mL). Cells that were cultured in medium alone served as a negative control. Cytokine levels were determined by analyzing 3-day culture supernatant by ELISA. Data are given as the mean ± the SEM. * $P < 0.05$ vs. ** by Student *t*-test.

Table 6. The levels of IFN-γ, IL-4, IL-6, IL-13, IL-10 and the ratios in the supernatant of non-irradiated peanut stimulated splenocytes cultures

	IFN-γ (pg/mL)	IL-4 (pg/mL)	IL-6 (pg/mL)	IL-13 (pg/mL)	IL-10 (pg/mL)	IFN-γ/IL-4 (ratio)	IFN-γ/IL-6 (ratio)	IFN-γ/IL-13 (ratio)	IFN-γ/IL-10 (ratio)
G1	1077.98	30.1	828.30	125.40	324.72	35.81	1.30	8.60	3.32
G2	3352.86	6.8	1707.18	155.90	358.24	493.07	1.96	21.51	9.36
G3	2652.18	15.42	541.65	163.41	249.38	172.00	4.90	16.23	10.64
G4	739.71	0	241.11	84.16	85.45	-	3.07	8.79	8.66

(G1: 0 kGy peanut intragastric, G2: 10 kGy peanut intragastric, G3 :50 kGy peanut intragastric, G4: Naive)

IV. DISCUSSION

In this study, the immunological properties of gamma-irradiated peanuts were evaluated in mice that had developed an immunogen leading to inhibition of the formation of the allergic response. Several studies conducted to evaluate the effects of gamma-irradiation on food allergens have reported that the gamma irradiated proteins were altered, which affected their intact antigenicity and IgE-binding ability due to the conformational modification induced by the gamma rays. In addition, studies have shown that irradiation can reduce or abolish the allergenicity of eggs, milk, wheat, and shrimp (Lee et al., 2002; Lee et al., 2001; Leszczynska et al., 2003; Byun et al., 2000). These results are similar to the results of the present study, in which stimulation of the splenocytes of mice that were allergic to peanuts and mice themselves by gamma-irradiated peanuts was found to increase the Th1 / Th 2 ratio and IL-10 production.

Unlike other allergic diseases, food allergy is the only disorder for which there is no specific therapy, which is particularly troubling considering the potential for severe reactions. Peanut allergy is the most serious of the hypersensitivity reactions to foods due to its persistence and high risk of severe anaphylaxis. Immunotherapy of peanut allergy using Chinese herbal medicines, probiotics, CpG-based immunotherapy and heat-killed *Listeria* expressing modified allergen therapy have all been studied (Nowak-Wegrzyn, 2006). Conventional immunotherapy attempted for peanut allergy could be used successfully to induce oral tolerance. However, the rate of serious adverse reactions was unacceptably high, even during the maintenance phase of immunotherapy (Nelson et al., 1997). Hence, many

studies for food allergy immunotherapy have been attempted. Allergic reactions are characterized by relative predominance of Th 2 type response t innocuous allergen. Most of the immunomodulatory therapeutic approaches to food allergy operate on the promise of restring the Th1/Th2 balance or activating regulatory T cells. In this study, for the first time the possibility for the reduction of peanut allergy by using a gamma-radiation technique is reported.

Ionizing radiation affects the properties of biomaterials by inducing physicochemical changes, and these effects are particularly notable in proteins (Garrison, 1987). Gamma-irradiated proteins maintain their immunological properties without the addition of novel immunlogical substances (Nascimento, 1996). In addition, the International Atomic Energy Agency (IAEA) concluded that an appropriate form of irradiation can keep food safe and wholesome without any adverse effects on human health (WHO, 1992; WHO, 1999). The safety of irradiated foods has been ensured by many federal agencies including the Food and Drug Administration (FDA), the U.S. Department of Agriculture (USDA), the Nuclear Regulatory Commission (NRC), the Occupational Safety and Health Administration (OSHA), and the Department of Transportation (USDOT).

However, to date, no studies have been conducted to evaluate the use of radiation for the prevention or treatment of allergies. Therefore, this study was conducted to investigate the immunological properties of the peanut, which is known to be a classical allergen, using a gamma irradiation in a mouse model. In addition, the potential for the role of the immunogen for treatment or reduction of allergies was assessed.

Gamma irradiation emits electrons and generates radicals as a result of the breakdown of the cobalt 60 isotope. The radicals then affect the intact protein structure by attacking the radio-sensitive residues or bond, which in turn induces fragmentation and aggregation of the protein. Radiolysis of the proteins primarily induces fragmentation and/or aggregation of the protein, as well as inactivation of the enzyme (Stevens et al., 1967; Saha et al., 1995; Audette-Stuart et al., 2005). In addition, Davies and Delsignore (1987) reported that irradiation of a protein under the presence of oxygen induced fragmentation and aggregation, and, it has also been reported that irradiation of proteins in a solution may change the secondary and tertiary structure due to the ease at which breakdown of the covalent bonds occurs, as well as the disruption of the ordered structure by the generated oxygen radicals (Lee et al., 2003).

In this study, SDS-PAGE and an immunoblotting assay revealed that fragmentation of the proteins had occurred, which shows that a structural change may reduce the binding capacity for the epitopes in ELISA, which is in agreement with the results of numerous other studies (Byun et al., 2000; Lee et al., 2001a; Lee et al., 2001b; Kim et al., 2002; Lee et al., 2002; Lee et al., Jeon et al., 2002, Seo, et al., 2004; Lee et al., 2005; Lee et al., 2007; Seo et al., 2007).

In addition, gamma-irradiated peanut bands was readily decreased or lost as a result of protease treatment, indicating the susceptibility of peanuts to protease digestion. The decrease or loss of irradiated peanut protein bands was also demonstrated by the fragmentation of peanut protein by irradiation. The general biochemical characteristics of most food allergens indicate that they are low molecular weight glycoproteins (< 70 kDa)

with acidic isoelectric points that are highly abundant in food. These proteins are usually resistant to protease, heat, and denaturants, which allows them to resist degradation during food preparation and digestion (Astwood et al., 1996; Deshpande et al., 1987). Resistance of allergens to digestive enzymes has been attributed to various factors, including the presence of protease inhibitors or nonprotein components present in the extracts analyzed (Desphande et al., 1987), direct effects on the secretion of endogenous proteins and/or the structure of the allergen itself (Baglieri et al., 1995). In addition, Maleki et al (2000) reported that the structure of the major peanut allergen, *Ara h* 1, may protect the IgE-binding epitope from digestive enzymes. In this study, the gamma-irradiation technique has allowed us to modify or destroy protease-resistant food allergen.

In addition, allergen immunotherapy that has been conducted using allergen extracts has focused on the use of modified allergens, including those in peptide form, polymerized allergens, or DNA vaccines that encode allergens (Larche and Wraith, 2005; Hayglass and Stefura, 1991; Li et al., 2005). Furthermore, radiation technology can induce conformational changes in proteins through the radicals generated by radiolysis (Davies and Delsignore, 1987). The results of a previous study indicated that gamma-irradiated allergens show the possibility of a novel immunogen for food allergy because the binding ability of sera obtained from patients with allergies for allergens that were structurally altered by gamma irradiation decreased (Byun et al., 2000; Lee et al., 2001; Part I).

When the mechanism by which the humoral immune responses by helper T cells occurs was considered, cytokines produced by Th1 and Th2 subsets were found to regulate the antibody producing action of B cells (Mosmann et al., 1986). Th1 cytokines such as IL-2 and

IFN-γ help B cells to produce IgG_{2a} type antibody, whereas Th2 cytokines (IL-4, IL-5, IL-6 and IL-13) differentiate and activate IgG1- and IgE-secreting B cells (Snapper et al., 1988). Specifically, IL-4 has been shown to lead to IgE and IgG_1 antibody class switching through germ line transcription of C_ε in humans and through $C_{\gamma 1}$ exons in murine B cells (Coffman et al., 1993).

When proliferation rate of the T cell was measured by ^{3}H-thymidine uptake assay at 72 h for a re-stimulation with 0 kGy non-irradiated peanut, 10 kGy irradiated peanut, and 50 kGy irradiated peanut on the spleen cells of the mice were allergic to peanut, T cells of the mice stimulated with 0 kGy non-irradiated peanut showed high rate of a proliferation. The spleen cells of the stimulated with 10 kGy-irradiated peanut were proliferated to a similar level as the 0 kGy non-irradiated peanut stimulation (P=0.286). In particular, that of the mice that stimulated with 50 kGy gamma-irradiated peanut significantly decreased with an increasing radiation dose. In intragastric administration model of irradiated peanuts, the activity of the group 2 that was administered with 10 kGy irradiated peanut were proliferated to a similar level as that of group 1 (P=0.127). While the activity of the group 3 that was administered with 50 kGy irradiated peanut extract was increased than that of group 1, which was not significant (P = 0.066). These result indicated that T cell stimulation activity of irradiated peanuts were not demolish by gamma-irradiation, which suggested that irradiated peanuts were partially satisfactory as immunogen capable of stimulation on T cell. So, we would speculate that less than 50 KGy gamma irradiation cause break the IgE epioptes and remain or increase the T cell epitopes. But, we did not confirm the higher dose effect of irradiation on the T cell epitope changes in this study.

Meanwhile, in vitro stimulation of gamma-irradiated peanuts in a culture of spleen cells obtained from peanut-allergic mice indicated that cytokine production appeared to be independent on the dosage of radiation. When cells were stimulated with 10 kGy irradiated peanut extracts, a higher level of production of both Th1-type (IFN-γ) and Th_{reg} type (IL-10) cytokines was observed. In addition, IL-6 production in response to 10 kGy peanut stimulation was higher than the production that occurred in response to non-irradiated peanut stimulation. Although these results indicate that 10 kGy irradiated peanut extract can partially induce Th 2 cytokine in cells, the IFN-γ /IL-6 ratio increased.

However, stimulation with 50 kGy irradiated peanut extract resulted in a higher level of production of only Th1-type (IFN-γ) cytokines. These results suggest that treatment with gamma irradiated peanut protein causes Th1-dominant peanut-specific immune responses. Baptista et al (2004) reported that IgG types of irradiated OVA were shown to switch Th 2 towards a Th 1 pattern, and in preliminary studies, we have had similar results were shown in a peanut allergic model that used C57BL/6 mice (data not shown). Taken together, these results indicate that the type of in vitro immune response to irradiated peanuts in mice is strain independent.

In this study intragastric administration of gamma-irradiated peanuts to the mice, peanut-specific cytokine productions appeared to be independent upon the irradiation dosage. For example, intragastric administration of 10 kGy irradiated peanuts elevated IFN-γ, IL-10, and IL-6 production. Conversely, intragastric administration of 50 kGy irradiated peanuts elevated only IFN-γ production and decreased IL-10 and IL-6 production. These phenomena were similar to part II data. These results indicated that epitopes of irradiated peanuts were

reformed newly and/or abolish particularly by the radiation dose. The irradiation of proteins at high doses is known to lead to denaturation as well as formation of protein radicals due to interactions with water radicals. In addition, low doses of irradiation can cause a very minor breakdown of food proteins into smaller molecular weight fragments and amino acids, whereas very high doses can cause cleavage of the amino acid side chains. On the other hands, optimal peptide loads for maximal cytokine productions can be different in individual cytokines, and sometimes, the kinetics of cytokine productions show concave or convex manner by increasing dosage, in this study, we could not find dose dependent patterns of production in several cytokines such as IFN- γ and IL-6. Furthermore, we would speculate that epitopes of 10 kGy irradiated peanut were formed newly by its proper radiation dose which appeared to have strong Th 1 immunomodulant effect. Conversely, the epitopes of 50 kGy irradiated peanut were abolish, which appeared to have weak Th 1 immunomodulant. Oligopeptides below a length of 8 amino acids are non-reactive with structures involved in antigen recognition or presentation, thus, are immunologically ignored (York et al., 1999). Also, change of the slope of the curve obtained from Ci-ELISA indicated the conformational alteration induced by irradiation of an epitope (Lee et al., 2001). Increase of the slope indicates the reduction of binding ability of antibody to protein. If the slope were similar, the epitopes were not likely to be altered in structure (Lee et al., 2001). The slopes of 10 kGy irradiated peanut were similar but those of 50 kGy irradiated peanut increased. Rupa and Mine (2006) showed that an engineered recombination of ovomucoid led to a disrupted structure that could be desensitized in allergic mice, which suggests that the deformation of an antigen can progress via a different mechanism. Gamma-irradiated peanuts showed

progression via a different mechanism than that of non-irradiated peanuts in the present mouse model.

Interestingly, intragastirc administration of 10 kGy irradiated peanuts elicited IL-10-secreting T cells. This elevation of IL-10 production, which occurred both in *in vitro* and in the administration model, indicates that T_{reg} cells are activated in these models. In immunotherapy, cytokines, such as IL-10 and TGF-β, are very important for maintaining the state of dynamic balance between Th1 and Th2 responses. Allergic reactions are characterized by the relative predominance of the Th2-type response to innocuous allergens, therefore most of the immunomodulatory therapeutic approaches to food allergy operate on the premise of restoring the Th1/Th2 balance or activating regulatory T cells.

Conversely, in the intragastric administration model of the 50 kGy irradiated peanut, IL-10 secretion from T cells was decreased, which indicates that the reduction of Th1 and Th2 response occurred as a result of T cell activation itself, which was similar to the results observed in Part II of this report.

Another important finding in the present study was that the Th1/Th2 ratio was found to increase in response to irradiated peanuts stimulation and administration, but not in response to non-irradiated peanut stimulation and administration. An increase in the Th1/Th2 ratio and a skewing of the allergen-specific effecter T cells to regulatory T (T_{reg}) cells appear to be a crucial event in the control of a healthy immune response to allergens and successful allergen-specific immune responses (Akidis et al., 2004). T_{reg} cells suppress the proliferation of allergen-induced specific T cells, cytokine secretion of both Th1 and Th2 type cells, and IgE production. Conversely, T_{reg} cells increase the production of IgG_4 and IgA antibodies and

secrete immunosuppressive cytokines such as IL-10 and TGF-β (Akidis et al., 2004; Jutel et al., 2003).

In addition, allergen-specific IgE plays a central role in food allergies and is an excellent target for interruption of the allergic process. In the 10 kGy irradiated peanut intragastric administration experiment, peanut-specific IgE production was not significantly different from that of the non-irradiated peanut administration group (P =0.221), which indicates that the possibility of the peanut-specific IgE binding epitopes reformed newly in 10 kGy irradiated peanut extract. Actually, as shown Fig. 3, 25 kDa band quantitation of 10 kGy irradiated peanut increased than that of 0 kGy non-irradiated peanut.

However, because this experiment was only conducted for a short amount of time, long term administration studies are required to evaluate the peanut-specific IgE reduction produced in response to treatment with irradiated peanut extract in a murine model of peanut allergy.

Conversely, the reduction of peanut-specific IgE production by irradiation with 50 kGy suggests that allergen modification through gamma irradiation may be applicable to the reduction and treatment of food allergies. Future studies are necessary to evaluate the proper radiation dose for reduction of peanut-specific IgE and elevation of IL-10.

Currently, the only therapeutic option available for the prevention of food allergic reactions is food avoidance. However, because peanuts are used as a protein supplement in a wide variety of processed foods, accidental consumption is almost inevitable. Therefore, gamma-irradiation for the treatment of allergens represents a promising tool for the treatment of food-hypersensitive patients. Characteristics of gamma-irradiated allergens that would

make them useful in a clinical setting include a reduced capacity to bind serum IgE, ensuring a lower risk of IgE-mediated anaphylactic side effects, and retention of their T-cell epitopes to allow modulation of the immune response.

Even though the many successful results from this study, we have several limitations. Especially we could not confirm the similarity between human and murine in vivo system. But we could understand the corresponding situation from a study by Li et al (2000). They reported that both T- and B-cell responses to the major peanut allergens in the mice are similar to those found in patient with peanut allergy and mouse IgE and human IgE bind to similar *Ara h* 2 epitopes and share the same immunodominant region. This immunologic similarity to human peanut allergy is an important feature because it will allow us to explore clinically relevant immunotherapeutic strategies using this result before launching similar studies in human subjects.

Taken together, the results of this study show the possibility of developing an immunogen capable of treatment and reduction of food allergy through h the use of gamma-radiation technology. Vaccination with gamma-irradiated peanuts can be applied to reduce peanut-induced allergy in a murine model of peanut allergy.

V. CONCLUSION

The binding ability of patients's IgE to irradiated peanut decreased depending on the dose. Also, SDS-PAGE and an immunoblotting assay revealed that fragmentation of the proteins had occurred, which shows that a structural change may reduce the binding capacity for the epitopes in ELISA. In murine experiment, that stimulation of the splenocytes of mice that were allergic to peanuts and mice themselves by gamma-irradiated peanuts was found to increase the Th1 / Th 2 ratio and IL-10 production. Therefore, this study showed the possibility of developing an immunogen capable of treatment and reduction of food allergy by using gamma-radiation technology. Future studies are required to further evaluate immunotherapy using irradiated peanuts in a murine model of peanut allergy.

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- 국문요약 -

땅콩 알레르기 생쥐 모델에서 면역치료를 위한 적절한 면역원으로서 감마선 조사된 땅콩 단백의 평가

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연구배경 및 목적 : 연구자들은 최근 난백에 감마선을 조사함으로 난백 알레르겐의 IgE 결합 epitope 의 구조를 변화시킴으로써 결국 난백의 알레르기 항원성을 감소시켜 난알부민 (ovalbumine, OVA) 을 이용한 난백 알레르기 환자를 대상으로 한 피부단자시험 (skin prick test) 에서 즉시형 피부반응의 격감효과를 증명한 바 있다. 이에 연구자들은 감마선 조사 기술을 땅콩 알레르기에도 적용하여 감마선 조사한 땅콩 단백과 조사하지 않은 땅콩 단백을 땅콩 환자혈청 및 기존에 확립한 땅콩 알레르기 생쥐 모델을 이용하여 감마선 조사 기술이 새로운 면역원 제조에 이용될 수 있는지를 평가하고자 하였다.

재료 및 방법 : 1) 땅콩에서 추출한 땅콩 단백에 각각 5, 10, 20, 50 kGy의 감마선을 조사한 후 감마선 조사하지 않은 순수 땅콩 단백과 함께 SDS-PAGE를 수행하여

단백 분획의 차이를 보았으며, 땅콩 알레르기 환자혈청을 이용하여 IgE immunoblot, ELISA, inhibition ELISA을 시행하여 순수 땅콩 단백과 감마선 조사된 땅콩 단백들에서의 땅콩 특이 IgE의 결합능을 비교 분석하여 항원성을 평가하였다. 또한, competitive indirect ELISA (Ci-ELISA)을 시행하여 감마선 조사된 땅콩 단백의 IgE, IgG 결합능을 순수 땅콩 단백과 정량으로 비교 분석하여 항원성을 평가하였다. 2) 4 주령의 암컷 C3H/HeJ 생쥐 10 마리를 각각 5마리씩 두 그룹으로 나눈 후 G1 (n=5)은 0 kGy 감마선 조사하지 않은 땅콩 단백을 항원보조제인 cholera toxin과 함께 각각 한 마리당 1 mg 씩 d0, 1, 2, 7에 위장 투여하여 감작시킨 후 d21에 challenge하였다. 이 때 G2 (n=5) 는 음성대조군으로 사용하였다. 감작 후 제 5 주에 생쥐를 희생하여 얻은 비장세포배양액으로부터 0 kGy 감마선 조사하지 않은 땅콩 단백, 10 kGy 감마선 조사한 땅콩 단백, 50 kGy 감마선 조사한 땅콩 단백으로 자극한 후 T 세포 증식분석과 사이토카인을 측정하여 감마선 조사된 땅콩 단백의 항원성을 비교 평가하였다. 3) 4 주령의 암컷 C3H/HeJ 생쥐 20마리를 각각 5마리씩 네 그룹으로 나눈 후 G1 (n=5)은 0 kGy 감마선 조사하지 않은 땅콩 단백을, G2 (n=5) 는 10 kGy 감마선 조사한 땅콩 단백을, G3 (n=5) 은 50 kGy 감마선 조사한 땅콩 단백을 항원보조제인 cholera toxin과 함께 각각 한 마리당 1 mg 씩 d0, 1, 2, 7에 위장 투여하여 감작시킨 후 d21에 challenge하였다. 이 때 G4 (n=5) 는 음성대조군으로 사용하였다. 감작시키는 동안 매주 생쥐의 미정맥으로부터 혈청을 분리하여 땅콩 특이 IgE, IgG_1, IgG_{2a} 를 측정하였으며 감작 후 제 5 주에 생쥐를 희생하여 얻은 비장세포배양액으로부터 T 세포 증식분석과 사이토카인을 분석하여 감마선

조사된 땅콩 단백의 항원성을 평가하였다.

결과 : 1) SDS-PAGE에서는 감마선 조사선량이 증가할수록 땅콩 단백 분획이 감소됨을 관찰할 수 있었으며, 땅콩 알레르기 환자의 혈청을 이용한 IgE immunoblot, ELISA, inhibition ELISA 실험에서 모두 감마선 조사선량이 증가할수록 땅콩 환자 혈청 내 땅콩 특이 IgE 의 결합능이 감소됨을 알 수 있었다. 또한 Ci-ELISA를 통해 감마선 조사로 인해 땅콩 단백의 구조가 변하였음을 관찰 할 수 있었다. 2) 땅콩 알레르기가 유발된 생쥐의 비장세포에 각각 0 kGy 감마선 조사하지 않은 땅콩 단백, 10 kGy 감마선 조사한 땅콩 단백, 50 kGy 감마선 조사한 땅콩 단백으로 자극하였을 경우, 감마선 조사한 땅콩 단백들 (10 kGy, 50 kGy)이 순수 땅콩 단백 (0 kGy) 의 자극보다 IL-10의 증가 및 Th1 / Th2 의 ratio 가 증가함을 알 수 있었다. 3) 생쥐에 순수 땅콩 단백 (0 kGy) 및 감마선 조사한 땅콩 단백 (10 kGy, 50 kGy) 을 위장 투여한 실험의 경우에서는 50 kGy 감마선 조사한 땅콩 단백을 위장 투여한 G3 에서 땅콩 특이 IgE가 감소함을 관찰할 수 있었으며, 땅콩 특이 IgG_1과 IgG_{2a}는 감마선 조사한 모든 그룹 (G2, 3) 에서 모두 감소하였다. 사이토카인의 경우 감마선 조사한 그룹 (G2, G3)에서 G1에 비해 IL-10의 증가와 더불어 Th1 / Th2의 ratio 가 증가함을 관찰할 수 있었다.

결론 : 감마선 조사선량이 증가함에 따라 땅콩 항원 이차구조에 변화가 일어났으며, 이로 인해 땅콩 알레르기 환자혈청 내 땅콩 특이 IgE 와의 반응성은 감소되었다. 또한 땅콩 알레르기를 유발한 생쥐의 비장세포 자극 모델에서는

감마선 조사된 땅콩단백의 자극이 순수 땅콩 단백에 비해 IL-10의 증가 및 Th 1 / Th 2 ratio 증가를 관찰되었다. 또한 감마선 조사된 땅콩 단백을 생쥐에 직접 위장 투여한 한 실험에서도 땅콩 특이 IgE의 감소와 더불어 IL-10의 증가와 함께 Th1 / Th 2 ratio가 증가함으로써 면역치료에 사용할 수 있는 새로운 면역원으로서의 긍정적인 평가를 할 수 있었다.

.핵심어 : 땅콩 알레르기, 생쥐모델, 감마선 조사, 사이토카인, 면역치료