

# Ovalbumin modified by gamma irradiation alters its immunological functions and allergic responses

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## Abstract

It is well known that gamma ( $\gamma$ )-ray irradiation results in the alteration of biological function of bioactive materials such as proteins, saccharides and lipids. In this study the effect of  $\gamma$ -irradiation on the chemical and immunological property of an allergen, ovalbumin (OVA), was investigated. Irradiation of more than 10 kGy resulted in the alteration of the structure of OVA. However, OVA treated with 10 kGy irradiation (10 kGy-OVA), but not 100 kGy-OVA, fully maintained immunological reactivity to a monoclonal antibody specific to the intact allergen (clone 14). Mice immunized with 10 kGy- as well as 100 kGy-OVA showed significantly lower antibody response to the allergen than those with intact OVA in a  $\gamma$ -ray dosage-dependent manner. Especially immunization of both 10 kGy- and 100 kGy-OVA induced a significant decrease of OVA-specific IgE. Splenocytes of mice immunized with irradiated OVA showed a significant reduction in OVA-specific T cell proliferation and the secretion of Th1-type (IFN- $\gamma$  and IL-2) and Th2-type cytokines (IL-4 and IL-6). The expression of T cell activation markers such as CD25 and CD44 was also down-regulated in T cells of mice immunized with irradiated OVAs. These results suggest that  $\gamma$ -ray irradiation of OVA suppress humoral and cellular immune responses specific to the allergen OVA, and the modification method with  $\gamma$ -irradiation may be available for the control of allergy.

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## 1. Introduction

Allergic response is caused by mediation of IgE antibody production against allergen from the sources of house dust mite, foods, animals, grass pollen etc. Induction of allergic reaction is associated with the complex mecha-

nisms such as increased production of allergen-specific IgE from B cells stimulated by IL-4, allergen-induced cross-linking of mast cell-bound IgE, the activation of eosinophils by secretion of IL-5, the release of inflammatory mediators, and Th2 cell-dominant immune responses [1]. Thus, down-regulation of Th2 responses is a logical approach for allergy immunotherapy. In addition many investigators have applied a variety of immunological trials to allergy treatment. Immunological tolerance and

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anergy against Th2 cells by increase of gradual administration of allergen or physically or chemically modified allergen, and reduction of Th2 cell response by suppression of Th2 response mechanism or usage of inhibitors for Th2-associated effector molecules (IL-4, IL-5, IL-13, and IgE) were shown to improve immunotherapy [2].

Meanwhile, ionizing radiation affects the property of physicochemical change of biomaterials, particularly proteins [3]. Irradiated proteins maintain the immunological property without addition of novel immunological substances [4]. The radiation technology to modify the property of biomaterials has been widely applied to detoxification of venom [4], reduction of IgE binding ability by modification of allergen [5,6], and manufacture of hypoallergenic cake [7]. However the application of radiation technology to prevention or treatment of allergy has not been tried yet. In this study we investigated the effect of  $\gamma$ -irradiation on the immunological properties of OVA, known to be a classical allergen, to induce humoral and cellular immune response specific to the allergen in mice.

## 2. Materials and methods

### 2.1. Gamma irradiation of allergen

OVA (ovalbumin, grade V, Sigma) was dissolved in a 0.01 M phosphate buffered saline (PBS) to 1 mg/ml. OVA solution was irradiated at 10 and 100 kGy in a cobalt-60 irradiator (IR-79, Nordion International Ltd.) equipped with 100 KCi activities and operated at a dose rate of 10 kGy/h. Gamma-irradiated OVA solutions were stored at 4 °C.

### 2.2. Sodium dodecyl sulfate–polyacryl amide gel electrophoresis (SDS–PAGE)

Electrophoresis was carried out using precast 4–20% Nu-PAGE Bis-Tris gels (Novex®, Invitrogen) at 100 V in a Nu-Page MES SDS running buffer (Invitrogen) system according to the manufacture's instructions. The gel was stained to visualize with Commassie Brilliant Blue R250.

### 2.3. Reactivity of intact and irradiated OVA to OVA-specific antibodies

Binding activity of intact and irradiated OVA to OVA-specific monoclonal and polyclonal antibody was measured by an immunoblot and enzyme-linked immunosorbent assay (ELISA) using mouse anti-OVA IgG monoclonal antibody (clone-14) (Sigma) and rabbit anti-OVA IgG polyclonal antibody. In immunoblot assay, protein bands of SDS–PAGE gel were electrotransferred to a PVDF membrane. The membrane was blocked by 2% bovine serum albumin (BSA) followed by washing with 0.05% Tween 20-Tris buffered saline (TBS). Clone-14 monoclonal antibody (mAb) or anti-OVA polyclonal IgG was added to the membrane [7]. Thereafter the membrane was reacted with goat anti-mouse IgG-horseradish peroxidase (HRP) (Southern Biotechnology Associates, Inc.) or goat anti-rabbit IgG-HRP (Sigma). The bands were visualized by diaminobenzene (DAB, Sigma) solution. In ELISA, the microtiter plates were coated with each OVA (10  $\mu$ g/ml) in 0.1 M bicarbonate buffer (pH 9.2) and incubated overnight at 4 °C. After blocking with 2% BSA, the serial dilutions of clone-14 mAb or anti-OVA polyclonal antibody were added to each well and incubated at 37 °C for 2 h. The wells were reacted with goat anti-mouse IgG antibody-HRP or goat anti-rabbit IgG-HRP, respectively. The plates were washed three times and developed by adding

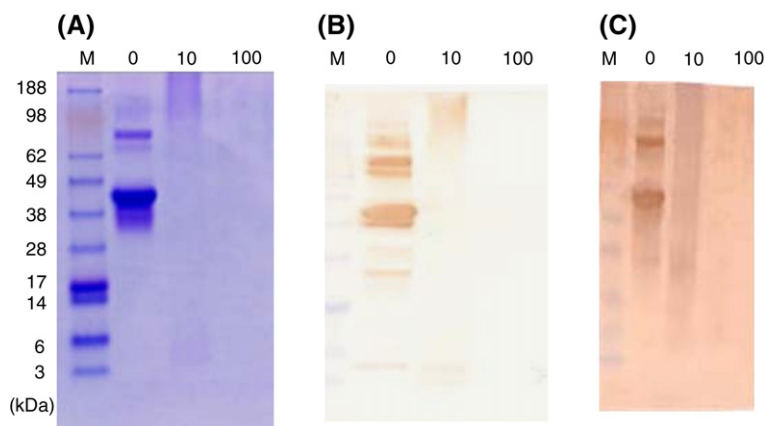


Fig. 1. The profiles of SDS–PAGE and immunoblot of intact and irradiated OVA. The indicated proteins (10  $\mu$ g/lane) was loaded to a 4–20% gradient gel in SDS–PAGE, and stained with Commassie Brilliant Blue R250 (A). Immunoblot was carried out using PVDF membranes as described in Materials and methods. The membranes were reacted with clone-14 mAb (B) or anti-OVA polyclonal antibody (C). “M” means a standard molecular marker.

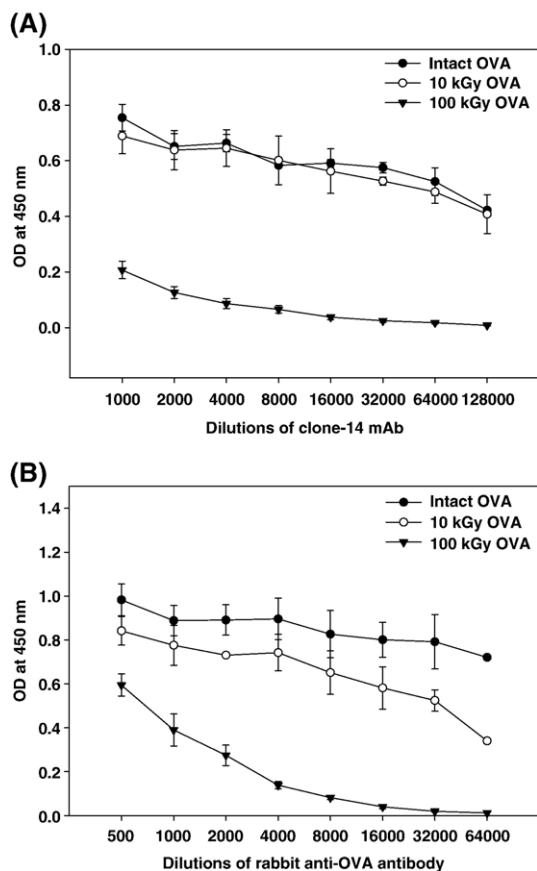


Fig. 2. Comparison of binding to OVA-specific antibodies between intact and irradiated OVA. Binding ability of the indicated OVAs to various dilutions of clone-14 mAb (A) or anti-OVA polyclonal antibody (B) was determined by ELISA.

3,3',5,5'-tetramethylbenzidine (TMB solution, Calbiochem). The absorbance of each well was read at 450 nm using a microtiter plate reader (Bio-Rad Laboratories).

#### 2.4. Immunization protocol

Groups of five BALB/c mice (female, 6 weeks-old) were immunized intraperitoneally (i.p) with 20  $\mu$ g of intact or irradiated OVA in 2.6 mg of aluminum hydroxide gel (alum, Sigma) on days 0 and 7. Two weeks after the last immunization, the mice were challenged with of intact OVA (20  $\mu$ g). Serum specimens of the mice were collected 1 week after intact OVA challenge.

#### 2.5. Determination of OVA-specific antibody responses

The microtiter plates were coated with intact or irradiated OVA (10  $\mu$ g/ml) and blocked by 2% BSA. Each well of the plate was reacted with the indicated dilutions of serum specimens obtained 1 week after intact OVA challenge, and incubated at 37 °C for 1 h. The wells were then incubated with rabbit anti-mouse IgG+IgA+IgM-HRP (Southern Biotechnology Associ-

ates, Inc.), and visualized by adding TMB solution. In assay for determination of subisotypes of OVA-specific immunoglobulins, biotinylated anti-IgG1, -IgG2a, -IgG2b, -IgG3, -IgM, and -IgE antibodies (BD Bioscience) at a 1:250 dilution were used as secondary antibodies. After wash, the plates were added by HRP-avidin D (Vector laboratories Inc.) at a 1:250 dilution. Visualization was conducted as described above.

#### 2.6. T cell proliferation assay

Splenocytes ( $1 \times 10^6$  cells/well) obtained from mice 1 week after intact OVA challenge were suspended in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin, and stimulated with intact OVA at a final concentration of 1.5–100  $\mu$ g/ml in a 96-well tissue culture plate for 72 h. T cell proliferation was measured by MTT (3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide) (Sigma) assay. Thirty microliters of MTT (5 mg/ml) dissolved in PBS was added into each well, and the

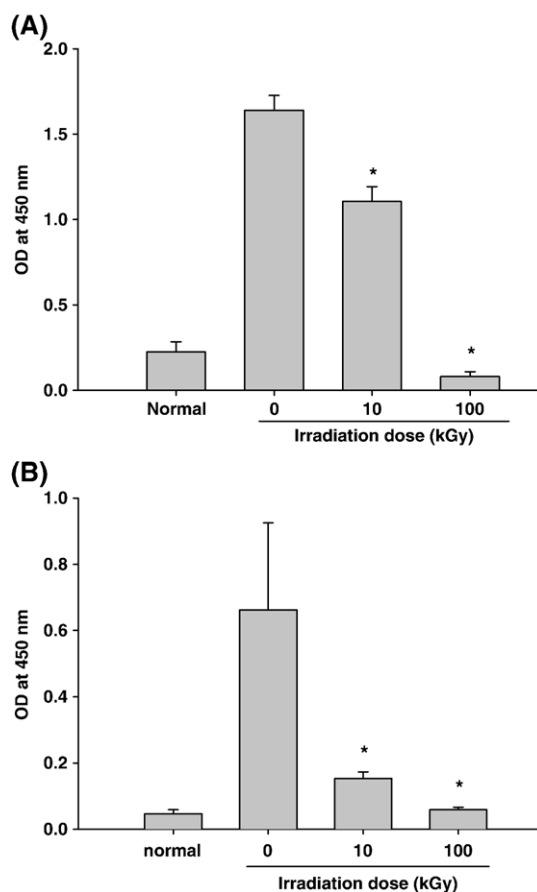


Fig. 3. Comparison of OVA-specific antibody-inducing activity between intact and irradiated OVA. The antibody titers to OVA-specific IgG+IgA+IgM (A) or IgE (B) were determined by ELISA as described in Materials and methods. \* $p < 0.001$ ; compared with intact OVA-immunized group (by student two-tailed *t*-test).

plate was incubated at 37 °C for 2 h. After centrifugation, the culture supernatants were removed and the cells were incubated with dimethylsulfoxide (DMSO, Sigma) (100  $\mu$ l) at 37 °C for 5 min. Absorbance of the control and OVA-treated cells was measured at 540 nm using a microplate reader.

## 2.7. Determination of cytokine levels

The culture supernatants obtained in T cell proliferation assay were used for determination of cytokine level. Cytokine level of the supernatants of splenocyte cultures was measured

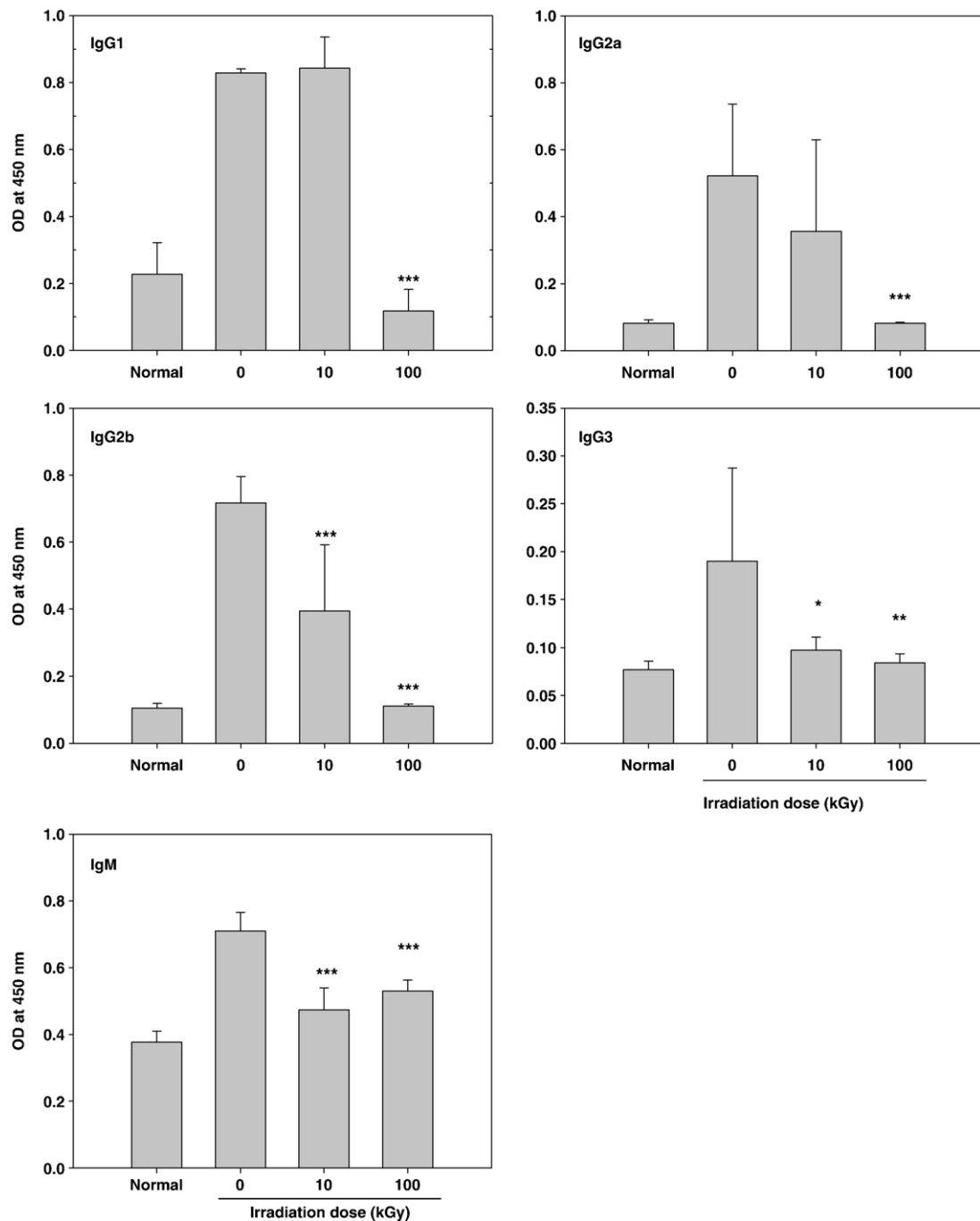


Fig. 4. Determination of subclasses of OVA-specific antibodies in serum specimens. Isotypes of OVA-specific antibodies were determined by ELISA using serum specimens obtained 1 week after intact OVA challenge. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ ; compared with intact OVA-immunized group (by student two-tailed  $t$ -test).

using IL-2, IL-4, IL-6, and IFN- $\gamma$  ELISA kits according to the manufacturer's instructions (BD Biosciences). Detection limits of IL-2, IL-4, IL-6, and IFN- $\gamma$  were 3.13, 7.8, 125, and 31.3 pg/ml, respectively.

### 2.8. Flow cytometry analysis

The level of T cell activation was examined by flow cytometry analysis using monoclonal antibodies (BD Bioscience) specific to activated T cell markers, CD25 and CD44. The splenocytes obtained from mice 1 week after intact OVA challenge were suspended in cold PBS containing 0.2% BSA and 0.1% NaN<sub>3</sub>. The cells were double-stained with the combination of fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse CD90.2 (Thy 1.2) mAb and R-phycoerythrin (R-PE)-conjugated rat anti-mouse CD25 (IL-2 receptor  $\alpha$  chain, p55) mAb, or the combination of FITC-Thy 1.2 mAb and R-PE-conjugated rat anti-mouse CD44 (Pgp-1, Ly-24) mAb. The stained cells were analyzed using a FACS Caliber flow cytometer equipped with CellQuest software (Becton Dickinson).

### 2.9. Statistical analysis

Statistical significance between the control and treated group was determined by two-tailed Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Chemical and immunological property of $\gamma$ -irradiated OVA

The effect of  $\gamma$ -irradiation on the chemical property of OVA was examined using SDS-PAGE. In electrophoresis, intact OVA showed two bands, 43 kDa major band and its dimmer of approximately 80 kDa (Fig. 1). However, these two bands were not found in either 10 kGy- or 100 kGy-OVA. It was also likely that irradiation with 10 kGy rendered a part of OVA to be large molecules with molecular weight of more than 90 kDa. In assay for reactivity of irradiated OVA to anti-OVA clone-14 mAb, 10 kGy-OVA was shown to be almost the same binding capacity to OVA-specific monoclonal antibody as intact OVA (Fig. 2). In contrast, 100 kGy-OVA had a low binding activity. This finding indicates that  $\gamma$ -irradiation with more than 10 kGy completely changes the structure of an allergen OVA, however, 10 kGy-OVA maintains immunological property to bind to OVA-specific monoclonal antibody.

### 3.2. Reduction of OVA-specific antibody production by $\gamma$ -irradiated OVA

To compare the activity to produce OVA-specific antibody between intact and  $\gamma$ -irradiated OVA, each protein was immunized twice and given intact OVA challenge. Their anti-sera were collected 1 week after intact OVA challenge for assay of

anti-OVA antibody response. Immunization of 10 kGy-OVA showed a significantly lower antibody response (OVA-specific IgG + A + M antibodies) comparing with intact OVA (Fig. 3A). In contrast 100 kGy-OVA could not produce OVA-specific antibody at all. Isotypes of OVA-specific antibodies raised by immunization of intact or  $\gamma$ -irradiated OVA were determined by ELISA. Fig. 4 shows that the lower activity of 10 kGy-OVA to produce OVA-specific antibody response was mainly related to low titers of IgG2b, IgG3 and IgM antibody specific to OVA. In consistent with the result of Fig. 3A, 100 kGy-OVA-immunized mice produced no OVA-specific antibody. Next we investigated the effect of allergen  $\gamma$ -irradiation on allergen-specific IgE production. As shown in Fig. 3B, mice that had been immunized with intact OVA and followed by challenge of the allergen produced high level of OVA-specific IgE antibody. In contrast mice immunized with both 10 kGy-OVA and 100 kGy-OVA showed a significantly lower OVA-specific IgE titers comparing with intact OVA-immunized mice. This data suggests that immunization of  $\gamma$ -irradiated OVA inhibit the production of OVA-specific IgE raised by challenge of the allergen.

### 3.3. Effect of $\gamma$ -irradiation on induction of allergen-specific cellular responses

The effect of  $\gamma$ -irradiation treatment on induction of OVA-specific cellular immune responses mediated by T cells was examined by an in vitro proliferation assay. The splenocytes obtained from mice immunized with intact or  $\gamma$ -irradiated OVA were co-incubated with the allergen OVA, and the proliferation of splenocytes was evaluated by MTT assay. As shown in Fig. 5, the splenocytes of mice immunized with 10 kGy-OVA as well as 100 kGy-OVA displayed a lower proliferating activity than those of mice immunized with intact

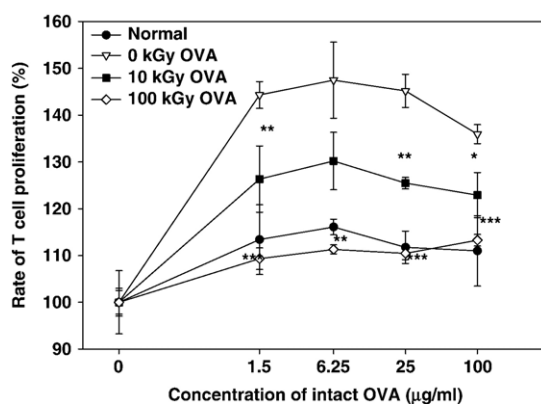


Fig. 5. T cell proliferating activity of the splenocytes of mice immunized with intact or irradiated OVA. Splenocytes obtained from mice 1 week after intact OVA challenge were co-incubated with the indicated doses of OVA for 72 h. The proliferative response was assessed by MTT assay. \**p*<0.05, \*\**p*<0.01, and \*\*\**p*<0.001; compared with intact OVA-immunized group (by student two-tailed *t*-test).

OVA. The activity of irradiated OVA to reduce OVA-specific T cell proliferation was strictly dependent upon the dosage of gamma irradiation. When we measured the level of cytokines secreted in the culture supernatants of splenocytes used in T cell proliferation assay, both  $\gamma$ -irradiated OVA-treated groups showed a markedly lower level of Th1 (IL-2 and IFN- $\gamma$ ) and Th2 (IL-4 and IL-6) type cytokines (Fig. 6). These results suggest that  $\gamma$ -irradiation of OVA suppresses its ability to induce antibody responses as well as cellular responses specific to the allergen protein.

### 3.4. Down-regulation of activated T cell markers in irradiated OVA-treated mice

To identify the level of T cell activation by intact or irradiated OVA, the expression of activated T cell markers such as CD25 (IL-2 receptor  $\alpha$ ) and CD44 were examined by FACS analysis. T cells treated with intact OVA showed higher expression of CD25 as well as CD44 than normal T cells (Fig. 7). However, the population of activated T cells expressing both activation markers (CD25<sup>+</sup> and CD44<sup>+</sup>) in irradiated OVA-treated mice was markedly down-regulated in a irradiation dosage-dependent manner. These results suggest

that the reduced activity of irradiated OVA to induce OVA-specific immune responses is related to the down-regulation of antigen-specific T cell activation.

## 4. Discussion

Gamma irradiation emits electron and generates radicals from the breakdown of the cobalt 60 isotope. The radicals affected the intact protein structure followed by an attack on the radio-sensitive residue or bond. Gamma irradiation to protein in an aqueous system induces a fragmentation and an aggregation. Davies and Delsigonoare [8] reported that the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions, and the formation of disulfide bonds from free radicals by a water-radiolysis might be induced by an aggregation of a protein to a higher molecular weight. We also found that gamma irradiation treatment to OVA resulted in the structural changes (Fig. 1) and immunological properties (Figs. 2 and 3) of this protein in SDS-PAGE and immunoassays,

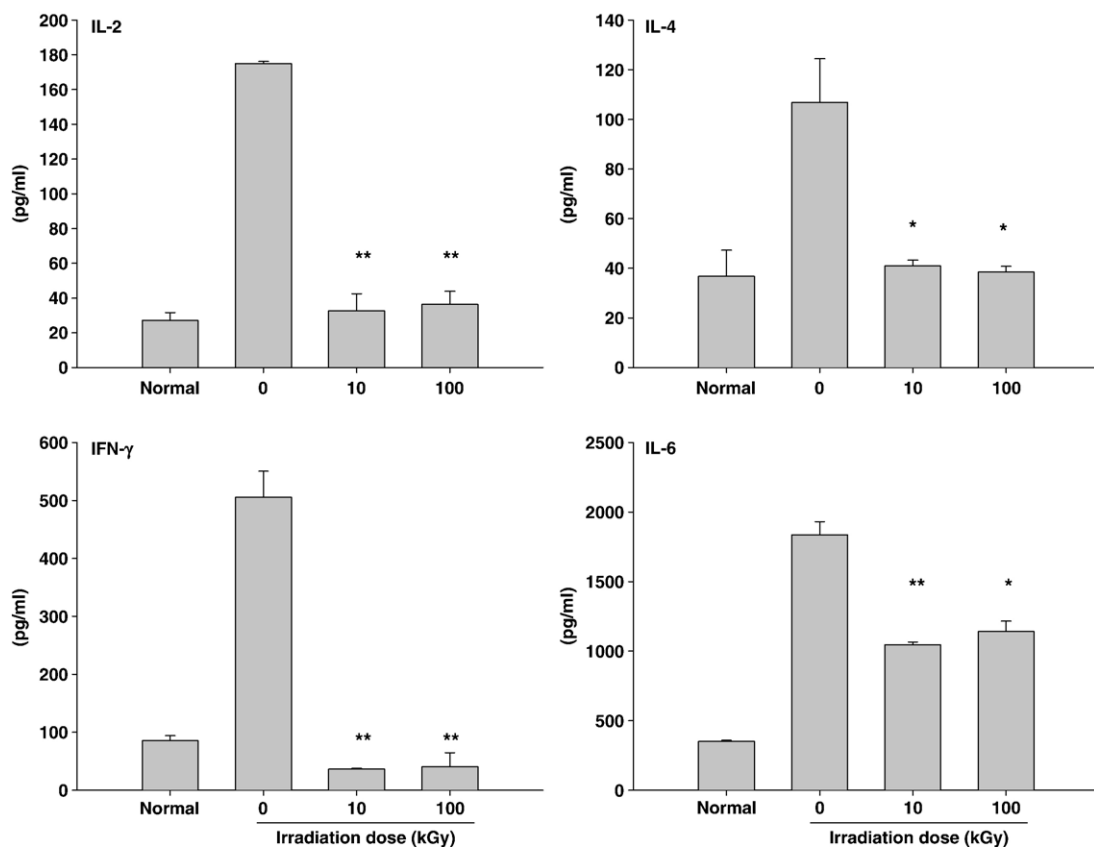


Fig. 6. Cytokine assay for IL-2, IL-4, IL-6 and IFN- $\gamma$  in the culture supernatants of splenocyte. The level of the indicated cytokines was measured using ELISA sets. \* $p < 0.05$  and \*\* $p < 0.01$ ; compared with intact OVA-immunized group (by student two-tailed  $t$ -test).

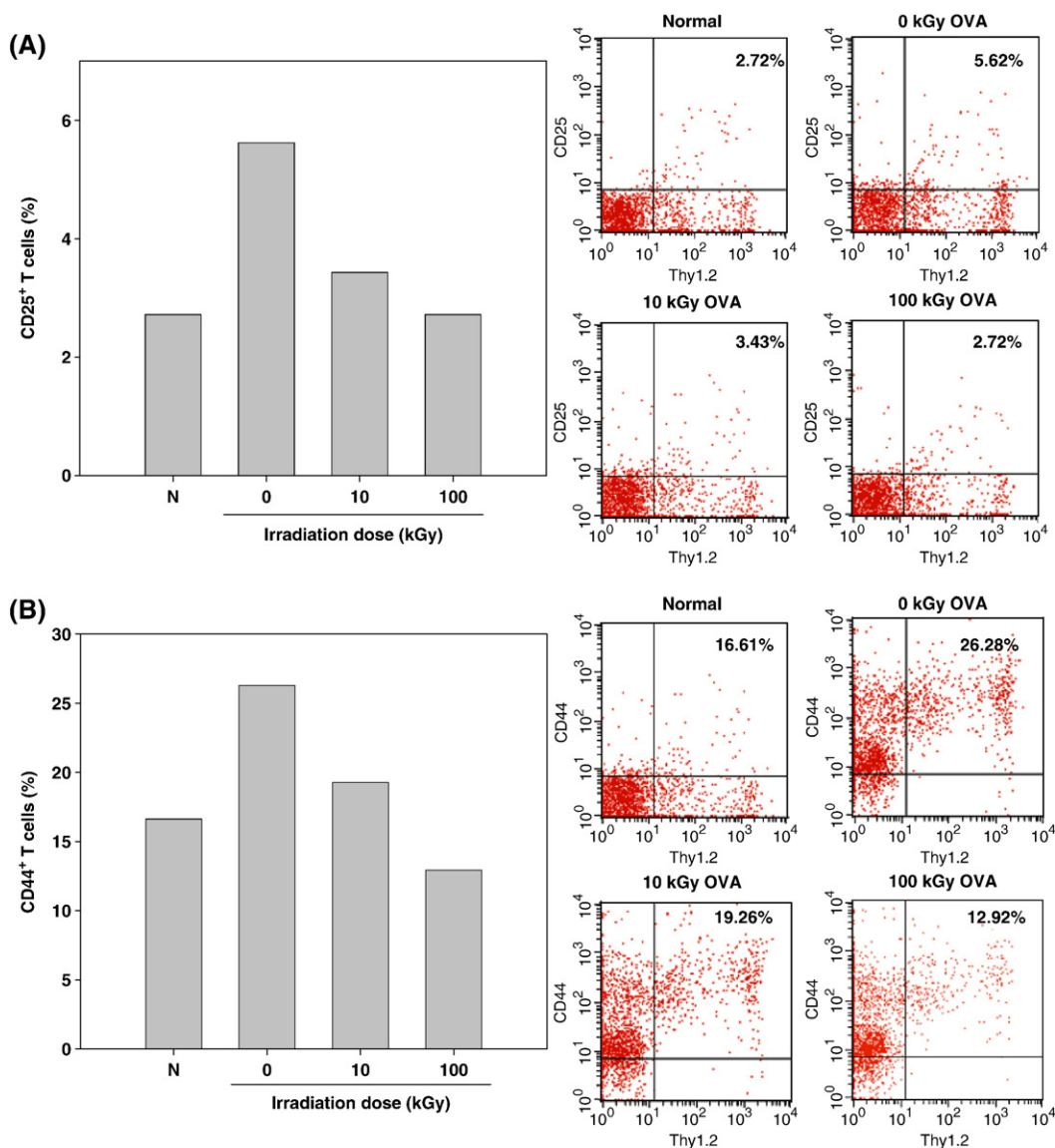


Fig. 7. FACS analysis for determination of activated T cell population. The population of CD25<sup>+</sup> (A) and CD44<sup>+</sup> (B) activated T cells was assayed by a flow cytometry using the splenocytes obtained from mice 1 week after intact OVA challenge.

respectively. The data is similar to the previous report suggesting that a structural change may reduce the binding capacity for the epitopes in ELISA [5,6]. Rupa and Mine [9] showed that an engineered recombination of ovomucoid leads to a disrupted structure and it can be desensitized in allergic mice. It suggests that the deformation of an antigen or allergen by irradiation treatment can elicit altered functions different from intact one.

Actually our previous studies demonstrated that various allergens such as ovalbumin, tropomyosin,  $\alpha$ -lactoglobulin,  $\beta$ -lactoglobulin, and casein structurally

modified by gamma irradiation reduced a binding ability to IgE of allergic patients [5–7,10]. Their properties might be applied to manufacture of functional foods for allergic patients [7]. The aim of this study is to investigate whether the treatment of gamma irradiation leads to the alteration in the immunological functions of the allergen OVA.

With regard to the regulatory mechanism of humoral immune responses by helper T cells, cytokines produced by Th1 and Th2 subsets regulate the antibody producing action of B cells [11]. Th1 cytokines such as IL-2 and IFN- $\gamma$  help B cells to produce IgG2a type antibody,

whereas Th2 cytokines (IL-4, IL-5, IL-6 and IL-13) differentiate and activate IgG1- and IgE-secreting B cells [12]. In particular IL-4 was shown to lead to IgE and IgG1 antibody class switching through a germ line transcription of  $C_\epsilon$  in a human and additionally  $C_{\gamma 1}$  exons in murine B cells [13]. When we examined OVA-specific antibody responses, mice treated with irradiated OVA showed a lower level of both Th1-type (IgG2a) and Th2-type (IgG1 and IgE) antibodies than those treated with intact OVA, and the reduction of antibody production in irradiated OVA seemed to be dependent upon irradiation dosage (Figs. 3 and 4). In an in vitro cytokine assay using the culture supernatants of splenocytes obtained from T cell proliferation test, it was observed that the splenocytes of mice immunized with irradiated OVA produced a lower level of both Th1-type (IL-2 and IFN- $\gamma$ ) and Th2-type (IL-4 and IL-6) cytokines (Fig. 6). These results suggest that gamma irradiation of OVA causes not only Th1-dominant but also Th2-dominant OVA-specific immune responses.

During the cascade of T cell activation, the cells stimulated by an antigen enhance the expression of activation markers such as CD25 and CD44 on the surface of them [14]. The data from the experiments of T cell proliferation (Fig. 5) and cytokine production (Fig. 6) indicate that immunization of irradiated OVA results in the suppression of T cell activation specific to the antigen OVA. So we examined the expression level of activation markers (CD25 and CD44) on the surface of T cells of mice immunized with intact or irradiated OVA. T cells treated with irradiated OVA showed a prominent reduction in the population of both CD25<sup>+</sup> and CD44<sup>+</sup> T cells compared to those immunized with intact OVA, and the down-regulation of activated T cell population seen in irradiated OVA-treated mice was dependent upon irradiation dosage (Fig. 7). Taken together, it is suggested that immunization of irradiated OVA induce just minimal to no activation of T cells, and the down-regulation of T cell activation by irradiated OVA may contribute to reduction of OVA-specific cellular and humoral immune responses.

An inhibition of the Th2-type response and a skewing of the allergen-specific effector 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 [15].  $T_{reg}$  cells suppress the proliferation of an allergen-induced specific T cells, cytokine secretion of both Th1 and Th2 type cells, and IgE production. On the other hand  $T_{reg}$  cells increase the production of the IgG4 and IgA antibody, and secrete immunosuppressive cytokines such as IL-10 and TGF- $\beta$  [15]. In our experiments,

however, immunization of irradiated OVA did not elicit IL-10-secreting T cells (data not shown). Thus it is likely that the reduction of OVA-specific immune responses induced by irradiated OVA is not associated with the  $T_{reg}$  cell response.

In the present study, we demonstrated that gamma irradiation of OVA caused a reduced humoral and cellular immune responses specific to the allergen OVA, and its related mechanisms were associated with down-regulation of OVA-specific T cell activation. Particularly, the inhibition of allergen-specific IgE production by irradiated allergen suggests the possibility that allergen modification method using gamma irradiation may be applicable to prevention and treatment of allergy. Further study to elucidate the immunological properties of irradiated allergens is currently underway.

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## References

- [1] Broide DH. Molecular and cellular mechanism of allergic disease. *J Allergy Clin Immunol* 2001;108:S65–71.
- [2] Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000;105:399–408.
- [3] Garrison WM. Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. *Chem Rev* 1987;87:381–98.
- [4] Nascimento ND, Seebart CS, Francis B, Roberto J, Kaiser II. Influence of ionizing radiation on crotoxin: biochemical and immunological aspects. *Toxicon* 1996;34:123–31.
- [5] Byun MW, Kim JH, Lee JW, Park JW, Hong CS, Kang IJ. Effects of gamma radiation on the conformation and antigenic properties of a heat-stable major allergen in brown shrimp. *J Food Prot* 2000;63:940–4.
- [6] Lee JW, Kim JW, Yook HS, Kang KO, Lee SY, Hwang HJ, et al. Effects of gamma radiation on the allergenicity and antigenicity properties of milk proteins. *J Food Prot* 2001;64:272–6.
- [7] Seo JH, Lee JW, Lee YS, Lee SY, Kim MR, Yook HS, et al. Change of an egg allergen in a white layer cake containing gamma-irradiated egg white. *J Food Prot* 2004;67:1463–8.
- [8] Byun MW, Lee JW, Seo JH, Kim JH, Jo C, Kim DH, et al. Changes of the immune reactivities of antibodies produced against gamma-irradiated antigen. *Radiat Phys Chem* 2004;71:127–9.
- [9] Davies KJ, Delsignore ME. Protein damage and degradation by oxygen radicals: III. Modification of secondary and tertiary structure. *J Biol Chem* 1987;262:9908–13.
- [10] Rupa R, Mine Y. Engineered recombinant ovomucoid third domain can desensitize Balb/c mice of egg allergy. *Allergy* 2006;61:836–42.
- [11] Mosmann TR, Cherwinski H, Bond MW, Giedli MA, Coffman RL. Two types of murine T cell clone, I: definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986;136:2348–57.

- [12] Snapper CM, Peschel C, Paul WEJ. INF- $\gamma$  stimulates IgG<sub>2a</sub> secretion by murine B cells stimulated with bacterial LPS. *J Immunol* 1988;140:2121–7.
- [13] Coffman RL, Lebman DA, Rothman P. Mechanism and regulation of immunoglobulin isotype switching. *Adv Immunol* 1993;54:229–70.
- [14] Krinzman SJ, de Sanctis GT, Cernadas M, Kobzik L, Listman JA, Christiani DC, et al. T cell activation in a murine model of asthma. *Am J Physiol* 1996;271:L476–83.
- [15] Akdis CA, Blaser K, Akdis M. Genes of tolerance. *Allergy* 2004;59:897–913.