



Effects of Red Ginseng extract on allergic reactions to food in Balb/c mice

Maho Sumiyoshi^a, Masahiro Sakanaka^a, Yoshiyuki Kimura^{b,*}

^a Division of Functional Histology, Department of Functional Biomedicine, Ehime University Graduate School of Medicine, Shitsukawa, Toon City, Ehime 791-0295, Japan

^b Division of Biochemical Pharmacology, Department of Basic Medical Research, Ehime University Graduate School of Medicine, Shitsukawa, Toon City, Ehime 791-0295, Japan

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ABSTRACT

Aim of the study: Red Ginseng roots (*Panax ginseng* C.A. Meyer) have traditionally been thought to have anti-allergic effects, but their influence on food-induced allergic responses is unclear.

Materials and methods: This study examined the effects of a Red Ginseng extract on an ova-albumin (OVA)-evoked allergic reaction in mice.

Results and conclusions: The orally administered extract significantly inhibited the increase in OVA-specific IgG₁ (Th₂) levels in OVA-sensitized mice, but had no effect on OVA-specific IgE (Th₂) levels. The extract prevented a reduction in IL-12 production and the ratio of IFN- γ (Th₁) to IL-4 (Th₂) in splenocytes, and enhanced small intestinal CD8-, IFN- γ -, and IgA-positive cell numbers in the OVA-sensitized mice. These findings suggest that Red Ginseng inhibits allergic reactions to food by preventing reductions in the ratio of IFN- γ to IL-4 and in IL-12 production induced by dietary antigens in spleen cells, and/or increasing the expression of CD8 and IFN- γ in the small intestine. It may also protect against sensitization to antigens as an immunomodulator by increasing intestinal IgA secretion without affecting antigen-specific IgE levels. In conclusion, Red Ginseng roots may be a natural preventative of food allergies.

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1. Introduction

Red Ginseng root (*Panax ginseng* C.A. Meyer) is used clinically in China, Korea, and Japan to treat atherosclerosis, liver dysfunction, cerebrovascular diseases, hypertension, post-menopausal disorder, and inflammatory allergic diseases (Yamamoto, 1998). Red Ginseng root extracts have also been used for the treatment of atopic suppurative dermatitis, wounds, inflammation of the skin, and allergic diarrhea. There are reports that ginseng saponins and/or extracts have anti-allergic properties including effects on asthma and dermatitis (Choo et al., 2003; Park et al., 2003, 2004; Shi et al., 2005; Bae et al., 2006; Shin et al., 2006a,b; Kim et al., 2008a,b; Bayayigit et al., 2008; Lim et al., 2009). Food allergies are common allergic diseases with a substantial risk of life-threatening anaphylactic reactions. Intestinal permeability is increased by allergic reactions to food, facilitating the absorption of the allergen into the circulation and leading to systemic allergic reactions (Perdue et al., 1984; Berin et al., 1997). However, effects of Red Ginseng extracts on allergic to food reactions are not yet proven. In this study, we examined the effect of a Red Ginseng extract on dietary allergic reactions including cytokine production in mice.

2. Materials and methods

2.1. Materials

Korean Red Ginseng Products are selected from ginseng carefully cultivated in well-fertilized fields for 6 years. The Ginseng extract, produced by Korea Ginseng Corporation (Taejon, Korea), was dried and powdered by freezing prior to use. The extract was analyzed by high-performance liquid chromatography (HPLC) (GLLIVER-HPLC System, JASCO Co., Tokyo, Japan) basically according to the methods of Hu et al. (2009) under the following conditions: monitoring wave length, 203 nm; flow rate, 1.5 ml/min; mobile phase, solvents (A) 0.1% trifluoroacetic acid (TFA) and (B) acetonitrile; gradient profile, 0–5 min 21% acetonitrile; 5–40 min 38% acetonitrile; column, TSK-Gel ODS-120T column (150 × 4.6 mm I.D.) (TOSO, Co., Tokyo, Japan); and column temperature, 30 °C. Ginsenoside standards (ginsenoside Rb₁, Rc, Rd, Re, and Rg₁) supplied by the late Prof. Dr. S. Arichi (The Research Institute of Oriental Medicine, Kinki University) were used for HPLC of the Red Ginseng extract. The purity of each standard was over 99.5%. The HPLC profile of the Red Ginseng extract is shown in Fig. 1.

Ova-albumin (OVA), concanavalin A (Con A), alum, bovine serum albumin (BSA), the biotinylation kit, and antibiotic and antimycotic solutions (100×) containing penicillin (10,000 U/ml), streptomycin (10 mg/ml), and amphotericin (25 µg/ml) were purchased from Sigma Co. (St. Louis, MO, USA). RPMI 1640 medium and fetal bovine serum (FBS) were obtained from Nissui Pharmacy Co. (Tokyo, Japan).

* Corresponding author. Tel.: +81 960 5922; fax: +81 89 960 5239.

E-mail address: yokim@m.ehime-u.ac.jp (Y. Kimura).

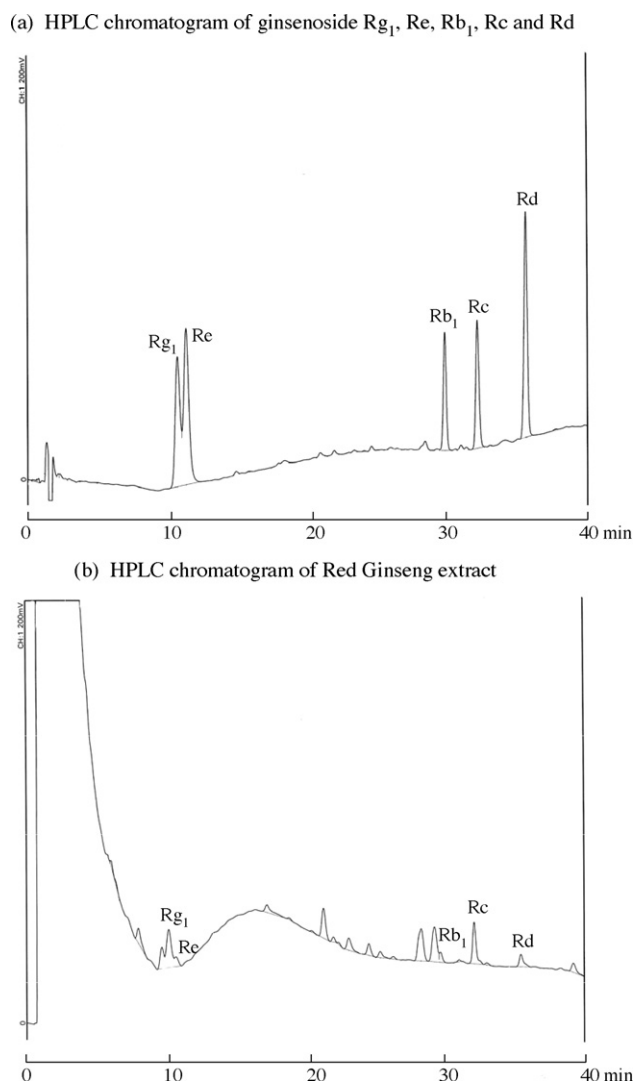


Fig. 1. HPLC of standard ginsenosides (Rb₁, Rc, Rd, Re and Rg₁) (a) and the Red Ginseng extract (b).

and GIBCO BRL (Auckland, New Zealand), respectively. Forty-eight-well culture plates and 96-well microtitre plates were purchased from Corning (NY, USA) and Becton Dickinson (NJ, USA), respectively. The biotinylated rabbit anti-mouse IgG and IgG₁ antibodies were purchased from Zymed Laboratories Inc. (CA, USA), biotinylated rabbit anti-mouse IgA antibody from Rockland (PA, USA), rat anti-mouse IgG_{2a} and IgE antibodies from Serotec (Oxford, UK), rabbit anti-mouse IgA antibody from Rockland (PA, USA), mouse anti-mouse CD8 α antibody from Santa Cruz (CA, USA), and rat anti-mouse IFN- γ antibody from PBL Biochemical Lab. (NJ, USA). Peroxidase-conjugated streptavidin and the 2,2'-azino-bis(3-ethyl-benzoline-6-sulfonic acid) (ABTS) substrate were obtained from DakoCytomation (Kyoto, Japan) and VECTOR Laboratories (CA, USA), respectively. The mouse interleukin (IL)-4 and interferon (IFN)- γ ELISA kits were bought from R & D Systems Inc. (MN, USA). The Mouse IL-12 ELISA kit was from Pierce Biotechnology Inc. (IL, USA). Mouse lymphocyte separation medium (Lymphocyte[®]-M) was obtained from Dainippon Pharmacy Co. (Osaka, Japan). The fluorescein isothiocyanate (FITC)-labeled anti-mouse CD4 and CD8 antibodies, phycoerythrin (PE)-labeled anti-mouse natural killer (NK) antibody, and FITC-labeled anti-mouse IgG_{2a} and PE-labeled anti-mouse IgG_{2a} (used as a negative control) were bought from Serotec (Oxford, UK). Other chemicals were of reagent grade and purchased from Wako Pure Chemicals Co. (Osaka, Japan).

2.2. Animals

The Balb/c mouse was used since it predominantly exhibits a Th2-type immune response after exposure to an allergen (Herz et al., 1998; Fukunaga et al., 2007; Finkelman and Wills-Karp, 2008). The C57BL/6 mouse exhibits a more Th1-like immune response (Herz et al., 1998; Fukunaga et al., 2007; Finkelman and Wills-Karp, 2008). Female mice were not used to avoid any effects of the sexual cycle on immune reactions. Male Balb/c mice (10 weeks old) were purchased from Clea Japan Co. (Osaka, Japan). They were housed for 1 week in a room with controlled temperature and humidity and given free access to food and water before the experiments were performed. The mice were treated according to the ethical guidelines of the Animal Center, Ehime University Graduate School of Medicine. The Animal Studies Committee of Ehime University approved the experimental protocol.

2.3. OVA-induced allergic reaction in mice

Male Balb/c mice (10 weeks old) were divided into four groups and given free access to a standard laboratory diet and water during the experimental period. The mice were sensitized with an intraperitoneal injection of OVA (3 μ g) with alum (2 mg) as an adjuvant on days 16 and 30 and then administered OVA (15 mg/kg body weight) orally on day 37. We previously reported that the oral administration of a Red Ginseng extract (20 and 60 mg/kg, twice daily) prevented ultraviolet B-irradiated skin damage in mice (Kim et al., 2008a,b). Generally, when administered to humans, Red Ginseng is taken three times per day at a dosage of 1000–3000 mg per 60 kg of body weight. Therefore, the extract was administered orally twice daily at a dose of 20 or 60 mg/kg body weight for 37 consecutive days to the OVA-sensitized mice. Normal and OVA-sensitized (control) mice were given distilled water alone according to the same schedule. Body weight and food intake were measured weekly. Blood samples were taken from each mouse by venous puncture under anesthesia with diethylether 24 h after the oral administration of OVA, and the mice were killed with an overdose of diethylether. Experiments were performed in a ventilated room. Serum was prepared and frozen at -20°C prior to analysis. The liver, spleen, thymus, and small intestine were removed from the body using sterile treatments, and the liver, spleen, and thymus were weighed.

2.4. OVA-specific immunoglobulin (Ig) levels in sera

To measure OVA-specific IgG, IgG₁, and IgA levels, 96-well microtitre plates were coated with 100 μ l of OVA at 200 μ g/ml in a 0.1 mM carbonate/bicarbonate buffer (pH 9.6) at 4°C overnight. Next they were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20, 150 μ l of PBS containing 1% BSA was added, and the plates were incubated for 2 h at room temperature. Following three washes with PBS containing 0.05% Tween 20, serum (100 μ l) diluted in PBS containing 1% BSA was added, and the plates were incubated for 2 h at room temperature. After another wash with PBS containing 0.05% Tween 20, biotinylated rabbit anti-mouse IgG, IgG₁, or IgA was added and incubation continued for 2 h at room temperature. The plates were then washed twice, and peroxidase-conjugated streptavidin was added. After further washing, the plates were incubated at room temperature with the ABTS substrate, and optical density (OD) was measured at 405 nm in a microplate reader (Nungle Nunc International, Tokyo, Japan). The assays of serum OVA-specific IgG_{2a} and IgE were performed as follows: briefly, 96-well microtitre plates were coated with the rat anti-mouse IgG_{2a} (10 μ g/ml) or IgE (5 μ g/ml) antibody in 0.1 mM carbonate/bicarbonate buffer (pH 9.6) at 4°C overnight. Subsequent procedures were performed using the meth-

Table 1Effects of Red Ginseng extract on body, liver, spleen, and thymus weights in mice sensitized with OVA^a.

Treatment	Body (g)	Liver (g)	Spleen (mg)	Thymus (mg)
Untreated mice (normal)	31.3 ± 0.46 ^b	1.774 ± 0.056 ^b	107.1 ± 6.95	32.3 ± 1.63
OVA-sensitized mice (control)	29.6 ± 0.58	1.622 ± 0.042	135.2 ± 7.15	33.6 ± 0.98
+ Red Ginseng extract (20 mg/kg, twice daily)	29.1 ± 0.63	1.572 ± 0.029	176.8 ± 17.30	32.0 ± 2.14
(60 mg/kg, twice daily)	29.3 ± 0.42	1.567 ± 0.055	192.9 ± 24.31 ^b	29.4 ± 1.80

^a Values are means ± SE for seven mice.^b Significantly different from OVA-sensitized mice (control), $P < 0.05$.

ods described above. After washing, 100 μ l of serum diluted in 10% inactive FBS and biotinylated OVA prepared using a biotinylation kit were added and incubated for 2 h at room temperature. Then the ABTS reagent was added and absorbance was measured using a microplate reader.

2.5. Cytokine production in splenocytes

The spleen was gently teased to release the cells in RPMI 1640 medium supplemented with 10% FBS, penicillin (100 U/ml), streptomycin (100 μ g/ml) and amphotericin B (0.25 μ g/ml), and the cell suspension was passed through a glass-wool column to remove cell debris and adherent cells. Isolated splenocytes were placed in RPMI 1640 medium containing 10% FBS, penicillin, streptomycin and amphotericin B at 2×10^6 cells per well in 48-well culture plates and incubated for 1 h. Con A (10 μ g/ml) was added and they were cultured for 48 h. The culture medium was centrifuged at 4 °C and $1500 \times g$ for 10 min to remove the cells. The supernatant was stored at –80 °C until analyzed for IL-4, IL-12, and IFN- γ , using mouse IL-4, IL-12, and IFN- γ ELISA kits.

2.6. CD4⁺ and CD8⁺ T cell and NK cell populations in splenocytes

The splenocyte suspension (5 ml) was layered on mouse lymphocyte separation medium (Lymphocyte[®]-M, 5 ml) and centrifuged at $1500 \times g$ for 30 min to isolate the lymphocytes. The number of lymphocytes was measured using a Coulter counter. The cell concentration was adjusted to 2×10^7 cells/ml, and 10- μ l volume of FITC-labeled anti-mouse CD4 and CD8 antibodies, PE-labeled anti-mouse NK, or FITC- and PE-labeled negative controls were added to 100 μ l of the cell suspension. After incubation for 30 min at 4 °C, the lymphocytes were rinsed three times with 1 ml of PBS and centrifuged at $700 \times g$ for 5 min. The CD4⁺ and CD8⁺ T cell and NK cell populations were immediately analyzed by flow cytometry using a FACS Calibur (Becton Dickinson, Mountain View, CA) without cell fixation.

2.7. Histological examination

All small intestines were fixed in 10% buffered formalin for at least 24 h, progressively dehydrated in solutions containing an increasingly higher percentage of ethanol (70, 80, 95 and 100%, v/v) cleared in HistoClear (FUME HOOD, As-ONE, Tokyo, Japan), embedded in paraffin under a vacuum, sectioned into 5- μ m-thick sections, de-paraffinized, and stained with hematoxylin and eosin

(HE). The CD8-, IFN- γ -, and IgA-positive cells in the small intestine were assessed by an immunoperoxidase technique using mouse anti-mouse CD8 α , rat-mouse IFN- γ , and rabbit anti-mouse IgA antibodies, respectively.

2.8. Statistical analysis

All values are expressed as means ± SE. Data were analyzed by one-way ANOVA, and then differences among means were analyzed using Fisher's protected test significance multiple-comparison test. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Effects of Red Ginseng extract on food intake, diarrheal incidence, and body, liver, spleen, and thymus weights in OVA-sensitized Balb/c mice

Food intake was decreased to 2.05 from 3.65 g/mouse/day (normal mice) by the first intraperitoneal injection of OVA, and to 1.48 from 3.23 g/mouse/day by the second injection. The decreases were not prevented by the oral administration of the Red Ginseng extract (data not shown). After the final sensitization using orally administered OVA, food intake did not differ between OVA-sensitized mice (control) and Red Ginseng extract-treated OVA-sensitized mice (data not shown). All mice of the control group had developed diarrhea by the final sensitization, and the diarrheal incidence was not reduced by the extract (data not shown).

There was no significant difference in final body or liver weight among normal mice, OVA-sensitized mice (control), and Red Ginseng extract-treated OVA-sensitized mice (Table 1). There was no significant difference in the weight of the thymus among normal, control, and Red Ginseng extract-treated mice. The spleen tended to increase in weight with the sensitization, although not significantly. The oral administration of the Red Ginseng extract (60 mg/kg, twice daily) significantly increased spleen weight compared to the control (Table 1).

3.2. Effects of Red Ginseng extract on serum OVA-specific immunoglobulin production in OVA-sensitized allergic mice

OVA-specific IgG, IgG₁, IgG_{2a}, IgA and IgE levels were increased by the sensitization (Table 2). The oral administration of the Red Ginseng extract significantly inhibited OVA-specific IgG₁ production in the OVA-sensitized mice. The extract had no effect on the

Table 2Effects of Red Ginseng extract on blood OVA-specific immunoglobulins (IgG, IgG₁, IgG_{2a}, IgA, and IgE) in mice sensitized with OVA^a.

Treatment	OVA-specific immunoglobulins (OD $\times$ 100)				
	IgG	IgG ₁	IgG _{2a}	IgA	IgE
Untreated mice (normal)	5.3 ± 1.1 ^b	6.0 ± 2.6 ^b	0.3 ± 0.1	20.5 ± 2.8 ^b	0.3 ± 0.2 ^b
OVA-sensitized mice (control)	81.0 ± 0.8	99.2 ± 0.7	1.5 ± 0.2	48.5 ± 4.8	4.4 ± 0.5
+ Red Ginseng extract (20 mg/kg, twice daily)	79.6 ± 0.5	96.6 ± 1.1	2.7 ± 0.7	47.2 ± 3.5	4.2 ± 0.7
(60 mg/kg, twice daily)	77.8 ± 0.9	94.8 ± 0.4 ^b	2.0 ± 0.5	49.3 ± 1.5	4.8 ± 0.8

^a Values are means ± SE for seven mice.^b Significantly different from OVA-sensitized mice (control), $P < 0.05$.

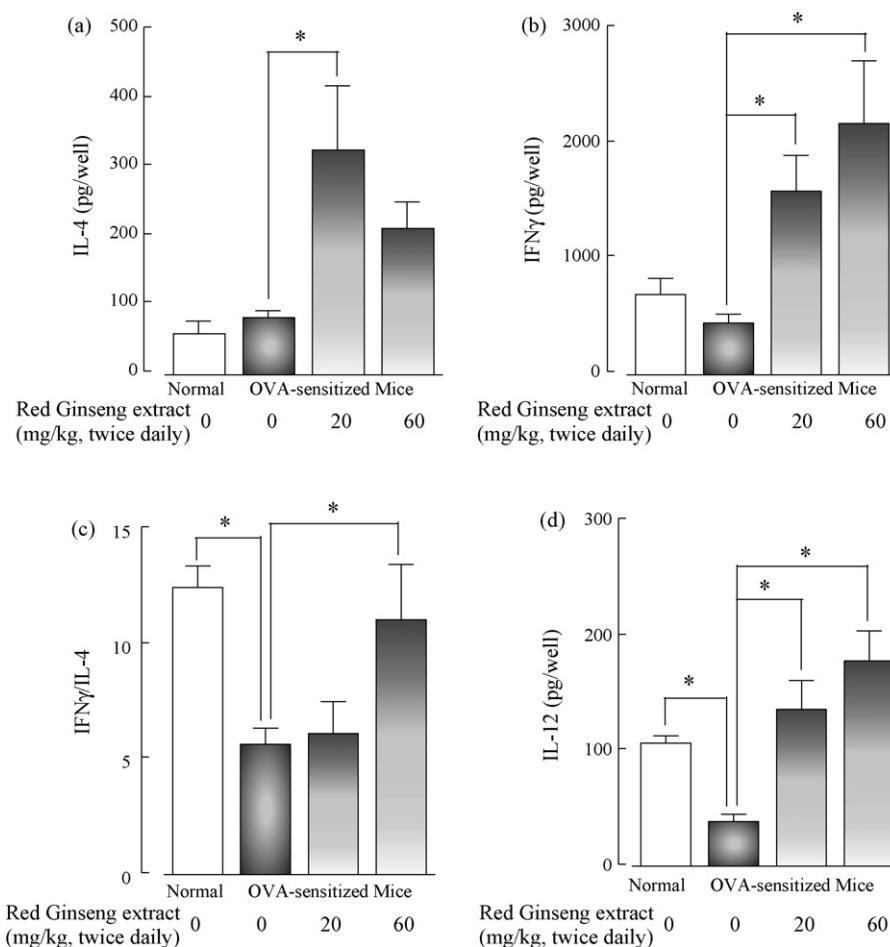


Fig. 2. Effects of Red Ginseng extract on Con A-induced IL-4 production (a), IFN-γ production (b), the ratio of IFN-γ/IL-4 production (c), and IL-12 production (d) by splenocytes in mice sensitized with OVA. Values are means $\pm$ SE for seven mice. *Significantly different from OVA-sensitized mice (control), $P < 0.05$.

increase in OVA-specific IgG, IgG_{2a}, IgE and IgA levels in the sensitized mice (Table 2).

3.3. Effects of Red Ginseng extract on cytokine production in spleen of OVA-sensitized allergic mice

There was no significant difference in Con A-induced IL-4 and IFN-γ production by splenocytes between normal mice and OVA-sensitized mice (control) (Fig. 2a and b). The Con A-induced production of IL-4 in the sensitized mice was significantly increased by the oral administration of the Red Ginseng extract at a dose of 20 mg/kg, but not 60 mg/kg. No dose-response was evident (Fig. 2a). On the other hand, the Con A-induced production of IFN-γ was significantly increased by the extract at 60 mg/kg (Fig. 2b).

The ratio of IFN-γ/IL-4 production was significantly lower in the OVA-sensitized mice than normal mice. The Red Ginseng extract (60 mg/kg) significantly inhibited the reduction in this ratio in the sensitized mice (control) (Fig. 2c). Con A-induced IL-12 production was significantly lower in the OVA-sensitized mice than normal mice. The oral administration of the extract (20 and 60 mg/kg) significantly inhibited the reduction in IL-12 production induced by the sensitization (Fig. 2d).

3.4. Effects of Red Ginseng extract on splenic immune functions in OVA-sensitized mice

There was no significant difference in the percentages of CD4⁺ T cells, CD8⁺ cells, and NK cells among normal mice, OVA-sensitized

Table 3

Effects of Red Ginseng extract on numbers of splenic lymphocytes, CD4⁺ T cells, CD8⁺ T cells, and NK cells in mice sensitized with OVA^a.

Treatment	Lymphocytes ($\times 10^5$ cells/spleen)	CD4 ⁺ T cells		CD8 ⁺ T cells		NK cells	
		($\times 10^5$ cells/spleen)	%	($\times 10^5$ cells/spleen)	%	($\times 10^5$ cells/spleen)	%
Untreated mice (normal)	88.3 $\pm$ 5.3 ^b	16.74 $\pm$ 1.24 ^b	19.05 $\pm$ 1.22	5.42 $\pm$ 0.89 ^b	6.12 $\pm$ 0.85	2.79 $\pm$ 0.17 ^b	3.21 $\pm$ 0.28
OVA-sensitized mice (control)	41.4 $\pm$ 3.9	9.15 $\pm$ 1.08	21.89 $\pm$ 1.24	2.80 $\pm$ 0.52	6.55 $\pm$ 0.86	1.37 $\pm$ 0.16	3.36 $\pm$ 0.31
+ Red Ginseng extract (20 mg/kg, twice daily)	41.8 $\pm$ 6.4	7.92 $\pm$ 1.19	19.14 $\pm$ 2.06	2.19 $\pm$ 0.55	5.12 $\pm$ 0.85	1.05 $\pm$ 0.13	2.62 $\pm$ 0.22
(60 mg/kg, twice daily)	63.6 $\pm$ 6.6 ^b	12.96 $\pm$ 0.88 ^b	20.85 $\pm$ 1.29	3.86 $\pm$ 0.37	6.19 $\pm$ 0.47	1.50 $\pm$ 0.15	2.44 $\pm$ 0.30

^a Values are means $\pm$ SE for seven mice.

^b Significantly different from OVA-sensitized mice (control), $P < 0.05$.

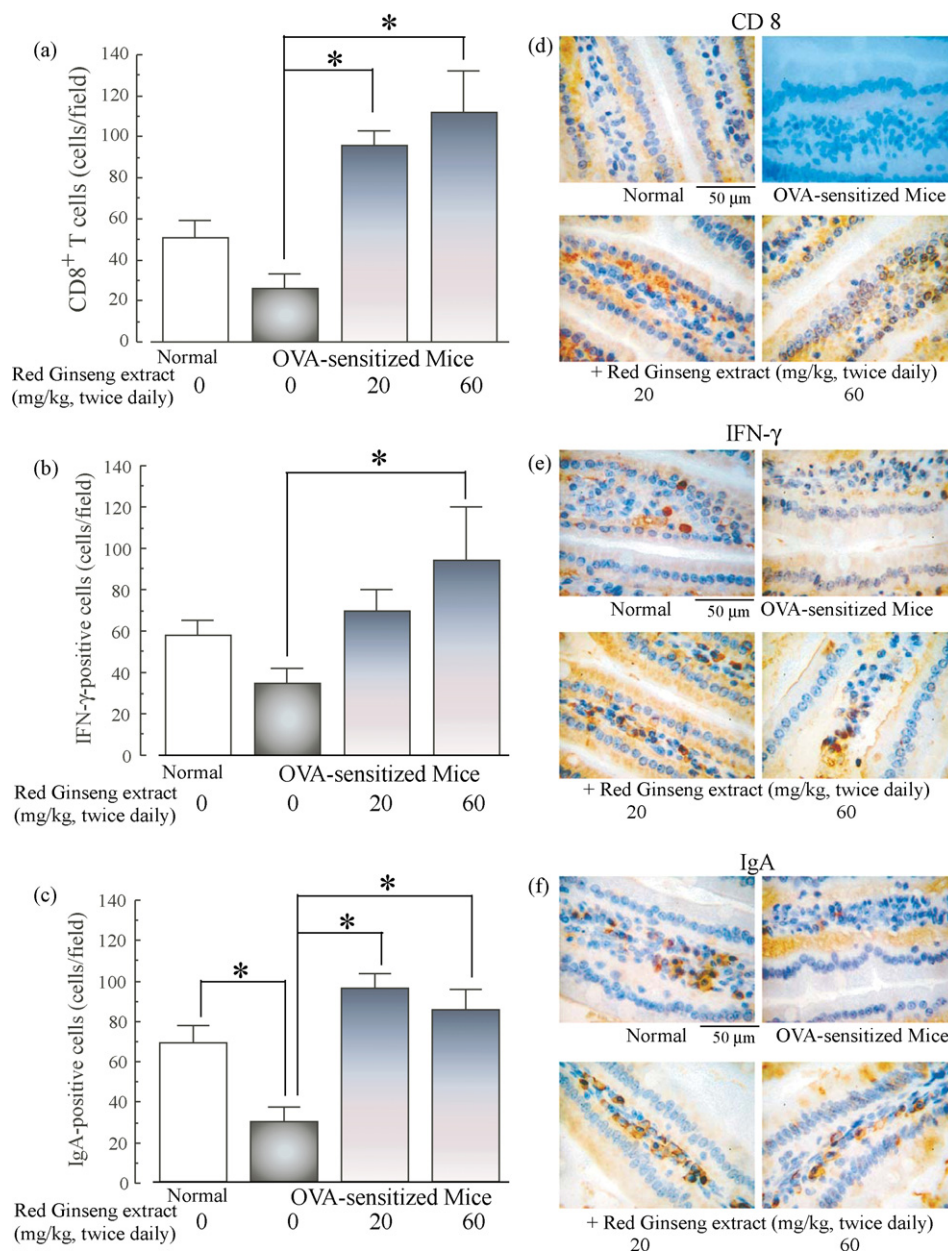


Fig. 3. Effects of Red Ginseng extract on CD8⁺, IFN-γ- and IgA-positive cells in the small intestine of mice sensitized with OVA. (a)–(c) Values are means ± SE for seven mice. *Significantly different from OVA-sensitized mice (control), $P < 0.05$. (d)–(f) Light micrographs of CD8⁺ T (d)-, IFN-γ (e)- and IgA (f)-positive cells in small intestine stained by the immunoperoxidase technique using mouse anti-mouse CD8α, rat anti-mouse IFN-γ, and rabbit anti-mouse IgA antibodies.

mice (control) and Red Ginseng extract-treated OVA-sensitized mice. However, numbers of splenic lymphocytes, CD4⁺ T cells, CD8⁺ T cells, and NK cells were significantly lower in the OVA-sensitized mice (control) than normal mice (Table 3). The oral administration of the Red Ginseng extract (60 mg/kg) significantly inhibited the reduction in lymphocytes and CD4⁺ T cells induced by the sensitization, but had no effect on the reduction in CD8⁺ T cells or NK cells (Table 3).

3.5. Effects of Red Ginseng extract on small intestine immune functions in OVA-sensitized mice

Although the numbers of CD8⁺ and IFN-γ-positive cells in the small intestine did not differ significantly between normal mice and OVA-sensitized mice (control), they were lower in the sensitized mice (Fig. 3a, b, d and e). The numbers of these cells were significantly increased by the oral administration of the Red Gin-

seng extract (20 or 60 mg/kg) in the OVA-sensitized mice (Fig. 3a, b, d and e). The number of IgA-positive cells in the small intestine was lower in the OVA-sensitized mice than normal mice (Fig. 3c and f). The orally administered extract (20 and 60 mg/kg) significantly inhibited this reduction (Fig. 3c and f).

4. Discussion

Food allergies are particularly common during childhood and may affect the final height and weight of a child (Christie et al., 2002). Furthermore, the incidence of these and other allergic diseases including allergic rhinitis and asthma is increasing in both developed and developing countries (Bruijnzeel-Koomen et al., 1995). Food allergies are most frequently the result of IgE-mediated hypersensitivity (Halgate, 1999). However, a number of food-related immunology-mediated gastrointestinal disorders

show no evidence of sensitivity to IgE (Sampson, 1991; Mori et al., 2009). The production of IgE is promoted by Th₂ cells and their cytokines, such as IL-4 and IL-5, but inhibited by IFN- γ secreted from Th₁ cells (Mosmann and Coffman, 1989). Furthermore, antigen-specific IgG_{2a} antibody production is induced by Th₁ cells and Th₁ cytokines, whereas IgE and IgG₁ antibody production is induced by Th₂ cells and Th₂ cytokines (Forsthuber et al., 1996; Abbas et al., 1997; Adkins and Du, 1998).

In the present study, we found that serum OVA-specific IgG, IgG₁, IgG_{2a}, IgE, and IgA concentrations were increased by sensitization with OVA. The increase in OVA-specific IgG₁ production induced by Th₂ cells and Th₂ cytokines was prevented by the oral administration of the Red Ginseng extract, but not that in OVA-specific IgE or IgA production.

The IgA in mucosal secretions is known to be protective of the mucosal immune system (Childers et al., 1989; Lamm et al., 1995). Takahashi et al. (1998) reported that lactic acid bacteria (*Bifidobacterium longum* OLL6001) stimulated cells of Peyer's patches, increasing secretory IgA antibody-related responses to dietary antigens in the small intestinal mucosa without harmful side effects, and suggested lactic acid bacteria to be effective as immunomodulatory agents in preventing systemic sensitization to dietary protein antigens. Furthermore, Frossard et al. (2007) reported that the oral administration of an IL-10-secreting *Lactococcus lactis* strain prevented serum IgE levels from rising and increased secretory IgA levels in the gut without affecting serum IgA levels in β -lactoglobulin-sensitized mice. On the other hand, small intestinal IgA levels were increased by the Red Ginseng extract. This finding suggests that the orally administered extract protects against sensitization to dietary antigens as an immunomodulator by increasing mucosal IgA secretion in the small intestine without affecting antigen-specific IgE levels. The mechanism by which Red Ginseng increases intestinal IgA levels remains to be clarified.

IL-12 is secreted by macrophages, monocytes, dendritic cells, and splenocytes in response to a variety of microbial factors (Hsieh et al., 1993; Macatonia et al., 1995; Shida et al., 2002). It plays a central role in the initiation of cell-mediated immunity both directly and through its induction of IFN- γ production (Chan et al., 1992; Seder et al., 1993), and induces the differentiation of Th₁ cells in CD4⁺ T cells, resulting in IFN- γ production by Th₁ cells, which enhances the Th₁-dominant response (Macatonia et al., 1995; Trinchieri, 1998). Furthermore, IL-12 plays an important role in the regulation of mucosal immunity (Marinero et al., 1997; Fass et al., 1999). In the present study, the orally administered Red Ginseng extract inhibited the reduction in splenic IL-12 production induced by sensitization to OVA. Furthermore, the ratio of IFN- γ /IL-4 production by splenocytes was reduced by the sensitization, and the extract inhibited this reduction. The number of splenic CD4⁺ T cells was also reduced by the sensitization with OVA, and the extract prevented this decline. These findings suggest that Red Ginseng may enhance the Th₁-dominant response by inhibiting the reduction in IL-12 levels induced by sensitization.

There are reports that intestinal intraepithelial lymphocytes (IELs) can secrete IL-2, IL-3, TNF- α , and IFN- γ (Taguchi et al., 1990; Barrett et al., 1992), and that the majority of IELs are CD4⁺CD8⁺ T cells consisting of two subpopulations, one expressing CD8 $\alpha\alpha$ homodimers and the other expressing CD8 $\alpha\beta$ heterodimers (Lefrancosis, 1991). IFN- γ was secreted from the IEL CD8 $\alpha\beta$ subpopulation in response to antigens derived from food and microorganisms (Steinhoff et al., 1999; Wang and Klein, 2001; Wang et al., 2002; Leav et al., 2005). In the current study, the Red Ginseng extract increased the numbers of small intestinal CD8⁺ T- and IFN- γ -positive cells.

Therefore, it seems likely that the orally administered extract inhibited the allergic reaction by preventing the reduction in IFN- γ /IL-4 and IL-12 induced by dietary antigens in the spleen cells,

and/or by increasing the numbers of intestinal CD8⁺ T- and IFN- γ -positive cells. In conclusion, Red Ginseng extract may be a natural preventative of food allergies.

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