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Amplified immune response by ginsenoside-based nanoparticles (ginsomes)

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ABSTRACT

We describe here a novel adjuvant of ginsenoside-based nanoparticles (ginsomes) and its activity for up-regulation of immune response in mice. Ginsomes were assembled during removal of the detergent by dialysis in presence of ginseng saponins extracted from the root of *Panax ginseng* C.A. Meyer, cholesterol and phosphatidyl choline. The nanoparticles were spherical with diameters ranging from 70 to 107 nm, and contained ginsenosides Rb2, Rc, Rb1 and Rd. When co-administered with a model antigen ovalbumin (OVA) in ICR mice, ginsomes at a dose range from 10 to 250 μg promoted significantly higher IgG responses than OVA alone. Co-administration of ginsomes with OVA also significantly increased the levels of specific IgG1, IgG2a, IgG2b and IgG3, as well as T and B lymphocyte proliferation in response to Con A, LPS and OVA than when OVA was used alone. The enhanced IgG titer and subclass levels paralleled the increased production of IFN-γ (Th1 cytokine) and IL-5 (Th2 cytokine). Therefore, ginsomes as an adjuvant have up-regulated both Th1 and Th2 immune responses.

1. Introduction

Vaccination remains the most cost-effective biomedical approach for the control of infectious diseases. The recombinant DNA derived subunit vaccines, while offering significant advantages over the traditional vaccines in term of safety and cost of production, generally represent isolated proteins or mixtures of proteins that have limited immunogenicity compared to whole microorganisms. These vaccines require addition of adjuvants to enhance their efficacy. Currently, the main hurdle to development of new and improved adjuvants has been safety, because vaccines that are to be used in healthy individuals will need to induce minimal adverse effects to prove acceptable for use. So, despite the description of over 100 adjuvants in the scientific literatures, aluminium salts (alum) are the only adjuvants used with vaccines licensed by the US FDA [1]. The limitation behind alum is that it is a poor inducer of Th1 cellular immune responses and stimulates the production of IgE antibodies, which is consistent with Th2 cellular immune response [2–4]. Unfortunately, a Th2-based immune response is not likely to offer optimal protection against several important infectious diseases, including TB, HIV and HCV.

Ginseng, the root of *Panax ginseng* C.A. Meyer, as a safe traditional medicine has been utilized in China for at least 2000 years [5]. The drug has been believed to stimulate the natural resistance against infections [6]. Recent studies on saponins extracted from the root of *P. ginseng* (GS) have demonstrated that GS has adjuvant activity capable of boosting both cellular (Th1) as well as humoral (Th2) immune responses. Rivera et al. [7] reported that co-administration of porcine parvovirus (PPV) antigens with GS has induced significantly higher haemagglutination inhibition (HI) titers than PPV antigens alone in guinea pigs. Hu et al. [8] reported that supplement of GS in a commercial *Staphylococcus aureus* bacterin has augmented specific antibody responses in cattle. Increased antibody responses have also been found in pigs when commercial vaccines against both PPV and *Erysipelothrix rhusiopathiae* infections were supplemented with GS [9]. More recent investigation has suggested that GS-supplemented vaccines elicit both Th1-like and Th2-like cytokine responses [10,11].

Early studies have demonstrated that the adjuvant potency may be amplified by formation of nanoparticles such as ISCOM matrix [12,13]. In this paper, we describe a nanoparticle (ginsomes) made from ginseng saponins, and evaluated it for adjuvant properties by measuring serum-specific antibody responses as well as lymphocyte proliferation and cytokine production by splenocytes in mice immunized with a model antigen ovalbumin (OVA) in combination with ginsomes.

2. Materials and methods

2.1. Ginseng extracts

Three ginseng extracts were used for screening out an optimal extract to make nanoparticles. They were normal ginseng saponins (GS-N) from the root of *P. ginseng* C.A. Meyer (Hongjiu Ginseng

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Co. Ltd., Jilin, China), stem–leaf ginseng saponins (GS-SL) from the stems and leaves of *P. ginseng* (Hongjiu Ginseng Co. Ltd., Jilin, China) and red ginseng saponins (GS-R) which were extracted using an ethanol-and-water procedure as described by Wang et al. [14] from the red ginseng roots, the product of Hongjiu Ginseng Co. Ltd. (Jilin, China). HPLC analysis showed that GS-N contained 3.0% Rg1, 5.2% Re, 8.5% Rb1, 9.8% Rb2, 12.7% Rc and 8.4% Rd, GS-SL 8.0% Rg1, 20.3% Re, 0.3% Rf, 1.4% Rb1, 3.7% Rc, 4.8% Rb2 and 11.9% Rd, and GS-R 0.9% Rg1, 1.9% Re, 1.2% Rf, 9.4% Rb1, 7.6% Rc, 6.6% Rb2, 6.8% Rb3 and 4.8% Rd.

2.2. Preparation of ginsenoside nanoparticles (ginsomes)

Preparation of ginsomes was mainly referred to the ISCOM matrix technology [15]. The ratio of ginseng saponin:cholesterol:phospholipid by weight and the reaction temperature for the formation of ginsomes were previously optimized. Among three ginseng extracts, only GS-R was found suitable to form ginsomes under the following described condition. Briefly, PC/C stock solution was prepared by dissolving phosphatidyl choline (150 mg) and cholesterol (150 mg) in 10 ml of 20% MEGA-10 (Ultra pure grade; Amresco, OH, USA) and kept at −20 °C until use. GS-R stock solution was prepared by dissolving GS-R powder in distilled water (100 mg/ml) and kept at −20 °C until use. PC/C (67 μl) and GS-R solution (200 μl) were mixed, and PBS (10 mM, 150 mM NaCl, pH 7.4) was added up to 1 ml of the total volume. The mixture was incubated with agitation every 20 min at 37 °C for 2 h. After that, the mixture was added into a dialysis tube with molecular weight cut-off 12,000, and dialyzed against PBS for 48 h.

2.3. Analysis of morphology and saponin components in ginsomes

Samples of ginsomes were applied onto carbon-coated grids, stained with 2% of phosphotungstic acid, and then envisaged under a transmission electron microscope (TEM) (JEM-1200EX, JEOL Ltd., Tokyo, Japan). For analysis of ginsenosides in ginsomes, ginsome components were separated using a chloroform–methanol–water procedure essentially as described by Behboudi et al. [16]. For analysis of individual ginsenosides, the saponin fraction was analyzed by HPLC (Analysis Laboratory, Hongjiu Ginseng Co. Ltd., Jilin, China). For analysis of total saponins in ginsomes, a colorimetric method as described by Behboudi et al. [16] was used with ginsenoside Rb1 as standard. To analyze the size distribution of ginsomes, 50 particles on the TEM digital image were measured by Adobe Photoshop 7.0.

2.4. Antigen–adjuvant preparation

Quil A extracted from the bark of *Quillaja saponaria* Molina was the product of NOR-VET ApS, Denmark. Solutions of ovalbumin (Endograde Ovalbumin, purity >98%, endotoxin concentration <0.5 endotoxin unit (EU)/mg; Profos AG, Germany) in 0.89% saline (100 μg/ml) with ginsomes containing total ginseng saponins of 2500, 500 and 100 μg/ml or with GS-R containing total ginseng saponin of 2500, 500 and 100 μg/ml or 500 μg/ml Quil A were prepared. The endotoxin level in each of the above solutions was less than 0.5 EU/ml evaluated by a gel-clot *Limulus* amebocyte lysate assay (Zhanjiang A & C Biological Ltd., Zhanjiang, China).

2.5. Animals

Six-week-old female ICR mice were purchased from Shanghai Laboratory Animal Center (SLAC) Co. Ltd. (Shanghai, China), and housed in polypropylene cages with sawdust bedding in hygienically controlled environment. Feed and water were supplied *ad libitum*. All procedures related to the animals and their care conformed to the internationally accepted principles as found in the Guidelines for Keeping Experimental Animals issued by the Government of China.

2.6. Immunization

In the first experiment, 35 mice were randomly divided into 7 groups of 5 animals. The mice were subcutaneously immunized twice at a 3-week interval with OVA (10 μg) in saline alone or with OVA in saline containing GS-R (10, 50, and 250 μg) or ginsomes (10, 50, and 250 μg). Sera were collected 2 weeks after the booster immunization for measurement of OVA-specific IgG levels.

In the second experiment, 32 mice were randomly divided into 4 groups of 8 animals each. Mice were subcutaneously immunized twice at weeks 0 and 3 with OVA (10 μg) alone as a control, or in combination with GS-R (50 μg), or Quil A (50 μg), or ginsomes (50 μg). Blood samples were collected from the orbital venous sinus at weeks 2 and 5 for measurement of serum OVA-specific IgG titers and the isotype responses. Splenocytes were harvested at week 5 for determination of lymphocyte proliferation and production of IFN-γ and IL-5.

All animals were anaesthetized hereafter prior to blood and spleen collection with ether. General behavior, appetite and local reaction at the injection sites were observed throughout the experiments and no adverse effect was found for the mice injected with OVA with or without ginsomes or GS-R or Quil A.

2.7. Determination of OVA-specific antibody

Serum OVA-specific IgG and the subclasses were measured by an indirect ELISA as described previously [11]. In brief, polystyrene 96-well plates were coated overnight at 4 °C with 100 μl of OVA solution (5 μg/ml in 50 mM carbonate–bicarbonate buffer, pH 9.6). The wells were washed three times with PBS containing 0.05% Tween-20 (v/v) (PBST) and then blocked with PBS containing 3% skimmed milk at 37 °C for 1 h. After three washes, 100 μl of serum (diluted 1:500 in PBS/1% skimmed milk for IgG level analysis or diluted 1:1000 for isotype analysis or diluted serially with initial dilution 1:20 for IgG titer analysis) was added to each well and incubated at 37 °C for 45 min. For IgG response detection, 100 μl of goat anti-mouse IgG (1:1000) (Kirkegaard, Perry Lab., MD, USA) was added to the wells and incubated at 37 °C for 45 min. Plates were washed again with PBST. A hundred microliters of 3,3′,5,5′-tetramethyl benzidine solution (100 μg/ml of 0.1 M citrate–phosphate, pH 5.0) was added to each well and incubated for 15 min at 37 °C. The reaction was stopped by adding 50 μl of 2 M H_2SO_4 to each well. The optical density of the plate was read by an automatic ELISA plate reader at 450 nm (Dialab, Austria). For subclasses, 100 μl of biotin-conjugated goat anti-mouse IgG1 or IgG2a or IgG2b or IgG3 (1:600) (Santa Cruz Biotechnology Inc., CA, USA) was added to corresponding plate and then incubated for 45 min at 37 °C. After washing, 100 μl of horseradish peroxidase-conjugated anti-biotin (BD Biosciences, Pharmingen, USA) diluted 1:4000 in PBS/1% skimmed milk was added to each well and incubated for 45 min at 37 °C. Incubations, washing and development were as described above for detection of OVA-specific IgG. The optical density of the plate was read at 450 nm. For OVA-specific IgG titer detection, values above the cut-off background level (mean value of sera from unimmunized mice multiplied by a factor of 2.1) were considered positive. Titers were depicted as reciprocal end-dilutions.

2.8. Splenocyte proliferation assay

Spleen collected from the OVA-immunized ICR mice under aseptic conditions, in Hank's balanced salt solution (HBSS, Sigma), was minced and passed through a fine steel mesh to obtain a homo-

geneous cell suspension. After centrifugation ($380 \times g$ at 4 °C for 10 min), the pelleted cells were washed three times in HBSS and resuspended in complete medium (RPMI 1640 supplemented with 0.05 mM 2-mercaptoethanol, 100 IU/ml penicillin, 100 μg/ml streptomycin and 10% heat-inactivated FCS). Cell numbers were counted with a haemocytometer by trypan blue dye exclusion technique. Cell viability exceeded 95%. Splenocyte proliferation was assayed as described previously [11] with some modification. Briefly, splenocytes were seeded into a 96-well flat-bottom microtiter plate (Nunc) at 5.0×10^6 cell/ml in 100 μl complete medium, thereafter concanavalin A (Con A, final concentration 5 μg/ml), LPS (final concentration 7.5 μg/ml), OVA (final concentration 10 μg/ml) or medium was added giving a final volume of 200 μl. The plates were incubated at 37 °C in a humid atmosphere with 5% CO_2 for 2 or 5 days. All the tests were carried out in triplicate. The cell proliferation was evaluated using MTT methods. Briefly, 50 μl of MTT solution (2 mg/ml) was added to each well 4 h before the end of incubation. The plates were centrifuged ($1400 \times g$, 5 min) and the untransformed MTT was removed carefully by pipetting. To each well 150 μl of a DMSO working solution (192 μl DMSO with 8 μl 1N HCl) was added, and the absorbance was evaluated in an ELISA reader at 570 nm with a 630 nm reference after 15 min. The stimulation index (SI) was calculated based on the following formula: SI = the absorbance value for mitogen cultures divided by the absorbance value for non-stimulated cultures.

2.9. IFN-γ and IL-5 release from splenocytes in vitro

Single cell suspensions were prepared from spleens colleted from the OVA-immunized mice under aseptic conditions and adjusted to a concentration of 2.5×10^6 cells/ml in RPMI 1640 supplemented with 2 mM L-glutamine, 50 mM 2-mercaptoethanol, 50 μl/ml gentamysin sulfate and 10% FCS. To a 96-well flat-bottom microtiter plate (Nunc), 100 μl of the cell suspension and equal volume of OVA solution (200 μg/ml) were added. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 72 h. After that, the culture supernatants were collected for cytokine assay. The concentrations of IFN-γ and IL-5 were determined by a commercial capture ELISA kit (R&D Systems Inc., Minneapolis, USA). Concentrations of cytokines were calculated from interpolation of the cytokine standard curve.

2.10. Statistical analysis

Data analysis was performed with SPSS software (SPSS, Version 11.5, SPSS Inc., Chicago, IL). One-way ANOVA analysis of variance followed by Bonferroni method of multiple comparisons was used to compare the parameters between groups [17]. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Morphology and ginsenosides of ginsomes

After negative staining with 2% of phosphotungstic acid, ginsomes were observed spherical under an electron microscope with diameters ranging from 87 to 100 nm with diameters ranging from 70 to 107 nm (87.4 ± 9.8 nm) (Fig. 1). A chloroform–methanol–water method was used to extract saponin in ginsomes. Analysis of the extract by HPLC showed that ginsomes contained ginsenosides Rb2, Rc, Rb1 and Rd but not Rg1, Re, Rf and Rb3 as detected in GS-R.

3.2. OVA-specific IgG and the isotype responses

Serum OVA-specific IgG and the subclasses were measured by an indirect ELISA to evaluate the adjuvant effect of ginsomes on the humoral immune responses. Fig. 2 shows that OVA-specific IgG levels in all ginsome-adjuvanted groups (10, 50 and 250 μg of ginsenosides in ginsomes) were significantly higher than those in the control or in all GS-R-adjuvanted groups (10, 50 and 250 μg of ginsenosides in GS-R) when the samples were collected 2 weeks after the last immunization ($P < 0.05$). Fig. 3 indicates that ginsomes (50 μg) triggered significantly stronger antibody responses than the OVA alone group at weeks 2 and 5, respectively ($P < 0.05$). As illustrated in Fig. 4, the levels of subclasses IgG1, IgG2a, IgG2b and IgG3 were also significantly higher in the mice immunized with OVA plus ginsomes (50 μg) than those of the mice immunized with OVA alone or with OVA plus GS-R (50 μg) ($P < 0.05$). However, no significant difference was observed for the responses of IgG1, IgG2a, IgG2b and IgG3 between GS-R and control groups. In Quil A-adjuvanted group, IgG2a level was significantly while IgG1 and IgG2b only were numerically higher than the control.

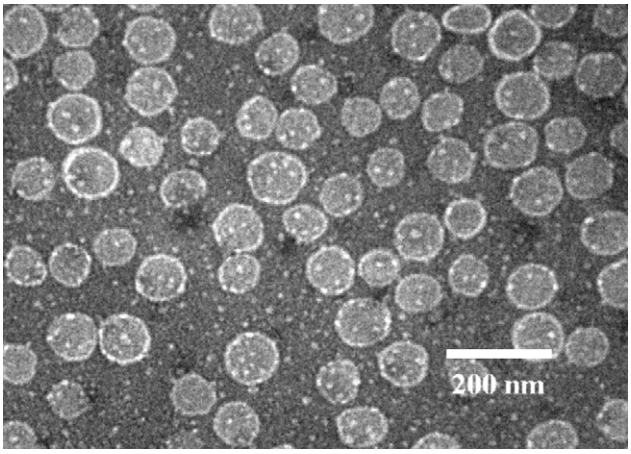

Fig. 1. Negative staining electron micrograph of ginsomes containing ginseng saponin, cholesterol and phosphatidyl choline (20:1:1, w/w/w). Ginsomes are spherical particles with diameters ranging from 70 to 107 nm (87.8 ± 9.4 nm).

3.3. Splenocyte responses

The effects of ginsomes on splenocyte proliferative responses to Con A, LPS and OVA stimulation are shown in Fig. 5. Mice immu-

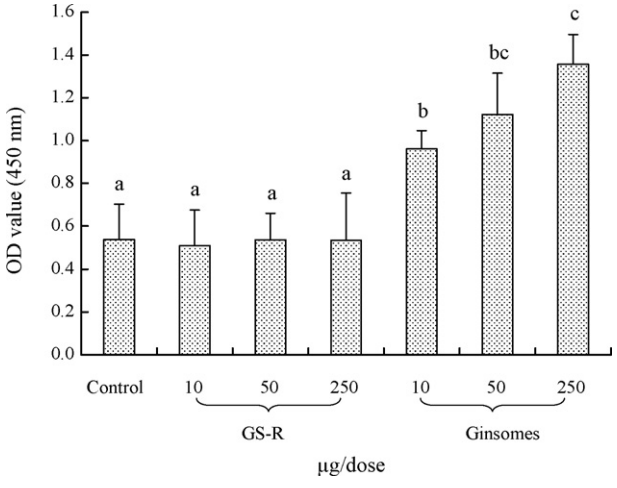

Fig. 2. Serum OVA-specific IgG responses. Mice were immunized s.c. twice at a 3-week interval with OVA (10 μg) alone as a control, or OVA (10 μg) + GS-R (10, 50 or 250 μg) or OVA (10 μg) + ginsome (10, 50 or 250 μg). Blood samples were collected for preparation of sera 2 weeks after the last immunization. OVA-specific IgG level was measured by an indirect ELISA as described in the text. Values represent means ± S.D. ($n = 5$). Bars with different letters are statistically different ($P < 0.05$).

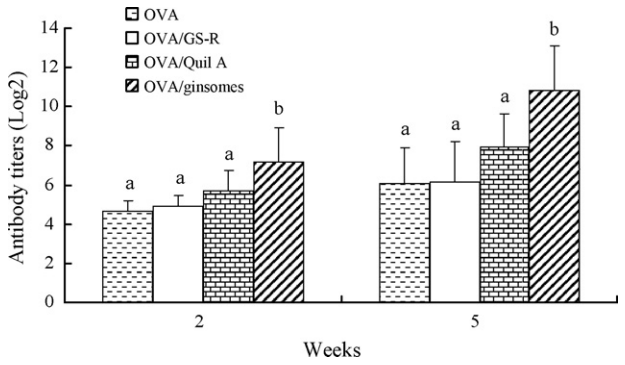

Fig. 3. Serum OVA-specific IgG titers at weeks 2 and 5. Six-week-old female ICR mice were subcutaneously immunized twice at weeks 0 and 3 with OVA (10 μg) alone as a control, or in combination with GS-R (50 μg), or Quil A (50 μg), or ginsomes (50 μg). Blood samples were collected at weeks 2 and 5. OVA-specific antibody titers (original dilution 1:20) were measured by an indirect ELISA as described in the text. Values above the cut-off background level (the mean from unimmunized mice as negative control multiplied by a factor of 2.1) were considered positive. Titers were depicted as reciprocal end-dilutions. Bars with different letters are statistically different ($P < 0.05$).

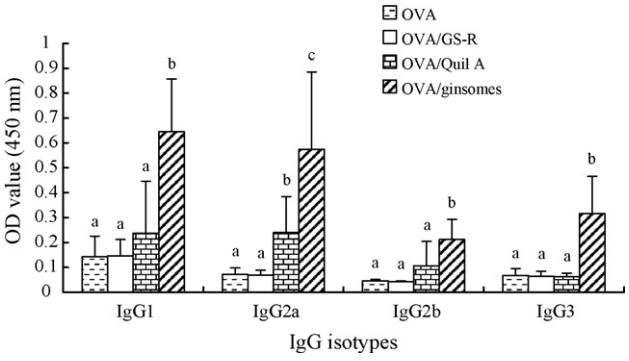

Fig. 4. Serum OVA-specific IgG1, IgG2a, IgG2b and IgG3 levels. Six-week-old female ICR mice were subcutaneously immunized twice at weeks 0 and 3 with OVA (10 μg) alone as a control, or in combination with GS-R (50 μg), or Quil A (50 μg), or ginsomes (50 μg). Blood samples were collected at week 5. OVA-specific antibody levels were measured by an indirect ELISA as described in the text. Bars with different letters are $P < 0.05$.

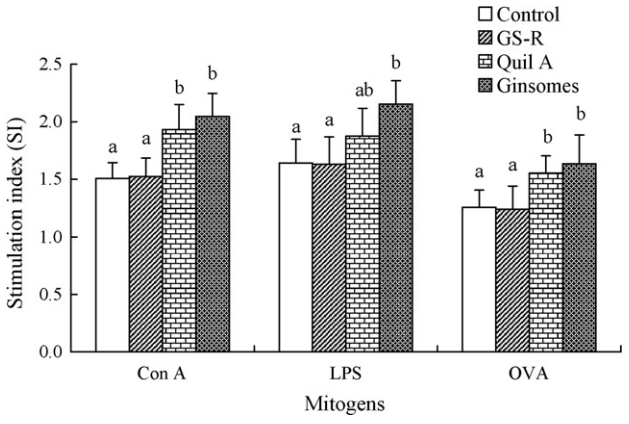

Fig. 5. Splenocyte proliferative responses to Con A, LPS and OVA. ICR mice were subcutaneously immunized twice at a 3-week interval with OVA (10 μg) alone as a control, or in combination with GS-R (50 μg), or Quil A (50 μg), or ginsomes (50 μg). Splenocytes were prepared 2 weeks after the last immunization and cultured with Con A (final concentration 5 μg/ml) or LPS (final concentration 7.5 μg/ml) or OVA (final concentration 10 μg/ml). Splenocyte proliferation was measured by the MTT method as described in the text, and shown as a stimulation index. The values are represented mean ± S.D. ($n = 8$ per group). Bars with different letters are $P < 0.05$.

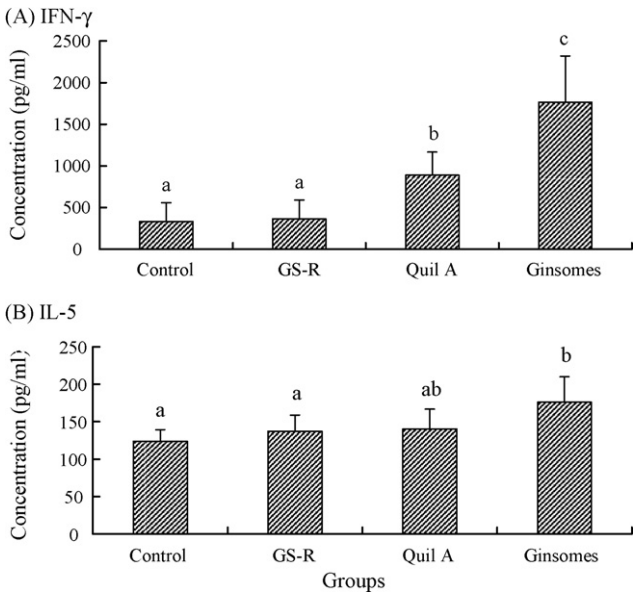

Fig. 6. IFN-γ (A) and IL-5 (B) production by splenocytes stimulated with OVA. Six-week-old female ICR mice were subcutaneously immunized twice at a 3-week interval with OVA (10 μg) alone as a control, or in combination with GS-R (50 μg), or Quil A (50 μg), or ginsomes (50 μg). Splenocytes were prepared 2 weeks after the last immunization, and cultured with OVA (final concentration 100 μg/ml). The culture supernatants were collected 72 h after stimulation. Values represent means ± S.D. ($n = 8$ per group). Bars with different letters are statistically different ($P < 0.05$).

nized with OVA plus ginsomes had significantly higher splenocyte proliferative response to Con A, LPS and OVA when compared to the mice injected with OVA alone ($P < 0.05$).

3.4. Production of IFN-γ and IL-5

After *in vitro* stimulation of splenocytes with OVA for 72 h, significantly higher production of both IFN-γ and IL-5 was detected in the cultures of the splenic cells from the mice immunized with OVA + ginsomes than that of the mice immunized with OVA alone (Fig. 6). When compared with the control, IFN-γ significantly but IL-5 only numerically increased in the mice immunized with OVA plus Quil A (Fig. 6).

4. Discussion

In preparation of ginsomes, on removal of the detergent (MEGA-10) by dialysis in presence of ginseng saponins, cholesterol and phosphatidyl choline, ginsomes assembly took place. Although the procedure for preparation of ginsomes is essentially the same as described by Lovgren-Bengtsson and Morein [15] for preparation of ISCOM matrix, ginsomes and ISCOM matrix are morphologically different. ISCOM matrix is a spherical and cage-like nanoparticle with a diameter of approximately 40 nm; ginsomes looks like nano-balls with diameters ranging from 70 to 107 nm. This morphological difference may be attributed to their different chemical structures of saponins used. The saponins for preparation of ISCOM matrix are extracted from the bark of the South American tree *Quillaja saponaria* Molina. They have a common structure consisting of the aglycone quillaic acid (3,16-dihydroxy-23-oxo-12-oleanen-28-oic acid) glycosylated at C-3 and C-28 [18,19]. Meanwhile, the saponins for making up ginsomes are ginsenosides extracted from the root of *P. ginseng* growing in the northeast China and Korea. They are triterpenoide glycosides sharing a dammarane skeleton as a common structure glycosylated at C-3 and C-20 (protopanaxadiol type) or at C-6 and C-20 (protopanaxatriol type) [20]. Nanoparticles are assumed to be assembled by hydrophobic interaction when

saponins, cholesterol and lipids are present in a system. Ginseng and Quillaja saponins have different structures which may influence the formation and result in different forms of nanoparticles. Not all kinds of ginseng saponins can be used to form nanoparticles. When GS-N, GS-SL and GS-R were used in present study, only GS-R was found having the property to form the particles. Such variation may be attributed to their different saponin profiles.

Although GS-R, as a raw material, contained ginsenosides Rg1, Re, Rf, Rb1, Rc, Rb2, Rb3 and Rd, only Rb2, Rc, Rb1 and Rd was detected in ginsomes by HPLC. Ginsenosides Rb2, Rc, Rb1 and Rd chemically belong to protopanaxadiol saponins while Rg1, Re and Rg2 to protopanaxatriol type [21,22]. Therefore, it seems that protopanaxatriol saponins have low affinity to cholesterol when compared with protopanaxadiol saponins, and they were removed during dialysis. However, it is still uncertain whether protopanaxadiol ginsenosides are essential for the formation of ginsomes.

Figs. 2 and 3 indicate that when co-administered with OVA, ginsomes at the doses ranging from 10 to 250 μg promoted significantly higher IgG levels than OVA alone. The enhanced IgG response persisted at least for 20 weeks (data not shown). There are different subclasses of IgG immunoglobulins such as IgG1, IgG2a, IgG2b and IgG3 that provide the bulk of immunity against most infectious agents. During a T cell-dependent immune response, there is a progressive change in the predominant immunoglobulin class of the specific antibody produced. This change, isotype switch, is influenced by T cells and their cytokines. In mice, IL-5 preferentially switches activated B cells to the IgG1 isotype (Th2 type immune response); IFN-γ enhances IgG2a and IgG3 responses (Th1 type); TGF-β (transforming growth factor-β) induces the switch to IgA or IgG2b [23]. Data in Fig. 4 indicates that co-administration of ginsomes with OVA significantly enhanced the responses of IgG1, IgG2a, IgG2b and IgG3. The enhanced IgG subclass responses may be explained by increased production of IFN-γ and IL-5 as indicated in Fig. 6. Therefore, ginsomes as an adjuvant have up-regulated both Th1 and Th2 immune responses. Whereas, Quil A used in this study mainly activated Th1-type immune responses with significantly higher IgG2a and IFN-γ production than the control, which is similar to the results as previously reported [15].

Data in Fig. 5 suggests that lymphocyte proliferative responses to Con A, LPS and OVA were enhanced in the mice immunized with OVA plus ginsome or Quil A. Increased lymphocyte response to Con A suggests that T lymphocyte were stimulated while enhanced response to LPS indicates that B lymphocytes were activated [24]. In order to induce antibody production, antigen-specific B lymphocytes should be triggered for clonal expansion. Enhanced lymphocyte responses to OVA stimulation paralleled the increased OVA-specific IgG response in the mice immunized with OVA plus ginsome.

Interestingly, free form of GS-R containing the same amount of ginsenosides as in ginsomes had no adjuvant effect on the antibody production, lymphocyte proliferation as well as cytokine release by splenocytes as indicated in Figs. 2–6 although GS-R is the raw material for composing ginsomes. The transformation of no adjuvant GS-R into adjuvant ginsomes may be due to facilitated antigen delivery caused by nano-scaled particles as seen in ISCOMatrix. But, no adjuvant effect of free form of ginseng saponins recorded in this study is contrary to our previous observations where significantly enhanced specific antibody responses have been detected when ginseng saponins were co-administered with antigens such as PPV antigens, *S. aureus* bacterin, *E. rhusiopathiae* antigens and OVA [7–10]. The discrepancy between GS-R and GS-N may be due to their different origins. GS-N originated from raw ginseng roots while GS-R from red ginseng roots. Red ginseng roots are traditional commercial products made from raw ginseng roots by steaming. During the steaming process, some chemical reaction may take place resulting in changes in saponin profile. In addition, antagonistic activity between saponin components in GS-R cannot be rule out. For example, Rivera et al. [7] observed that the antibody titer was significantly lower when Rb1 and Rg1 were included in the same dose of vaccine than Rb1 was used alone.

In conclusion, ginsomes assembly took place in presence of GS-R, cholesterol and phosphatidyl choline, during dialysis to remove the detergent (MEGA-10). Ginsomes are nanoparticles with diameters ranging from 50 to 100 nm, and contained ginsenosides Rb2, Rc, Rb1 and Rd. When co-administered with OVA in ICR mice, ginsomes at a dose range from 10 to 250 μg promoted significantly higher IgG levels than OVA alone. Co-administration of ginsomes with OVA has also significantly increased the levels of IgG1, IgG2a, IgG2b and IgG3, as well as T and B lymphocyte proliferation in response to Con A, LPS and OVA than when OVA was used alone. The enhanced IgG subclass levels paralleled the increased production of IFN-γ and IL-5 in the cell cultures of the mice immunized with OVA + ginsomes. Therefore, ginsomes as an adjuvant have up-regulated both Th1 and Th2 immune responses.

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