Intraperitoneal Injection of Ginseng Extract Enhances Both Immunoglobulin and Cytokine Production in Mice

Chian-Jiun Liou
Kang-Ning Junior College of Medical Care and Management
Nei-Hu, Taipei, Taiwan

Ming-Liang Li
Department of Biology, National Taiwan Normal University
Taipei 116, Taiwan

Jerming Tseng
Department of Medical Education and Research, Changhua Christian Hospital
Changhua 500, Taiwan

Abstract: Ginseng is one of the most widely used Chinese herbal medicines. In this report, the relatively short-term effect of ginseng extract on the immunoglobulin production and cytokine production was studied. The ginseng extract was prepared by boiling the ground ginseng root in 50% ethanol. The specific pathogen-free mice were intraperitoneally (i.p.) injected with various doses of ginseng extract for 3 consecutive days. The results indicated that the serum levels of immunoglobulin (Ig) M, IgG and IgA were significantly elevated after the mice were i.p. injected with 4 g/kg/day of ginseng extract. Under *in vitro* condition, the lipopolysaccharide (LPS)-stimulated spleen cells showed a dose-dependent increase in secretion of IgM, IgG and IgA. However, at a higher dosage (4 g/kg/day), the amount of IgA secretion began to decline. The serum level of interleukin (IL)-2, interferon (IFN)-γ (T-helper (Th)1-type cytokines) and IL-4 and IL-10 (Th2-type cytokines) were significantly elevated after the mice were i.p. injected with 2 g/kg/day or higher doses of ginseng extract. The amount of cytokine secretion by concanavalin A (Con A)-stimulated spleen cells was also significantly enhanced after the mice were i.p. injected with 0.4 g/kg/day or higher dose of ginseng extracted. To further confirm the results from enzyme-linked immunosorbent assay (ELISA), the spleen cells were cultured for 36 hours in the presence of 1 µg/ml of Con A. Total mRNA was isolated and assayed for mRNA expression using reverse transcriptase-polymerase chain reaction (RT-PCR). The results revealed that expression of IL-2 and IFN-γ mRNA were dose-dependently enhanced by the ethanol extract of ginseng. The levels of IL-4 and IL-10 mRNA expression
were also elevated in the spleen cells of ginseng-treated mice in comparison with that of the control group. In addition, we observed that the concentrations of IgG1, IgG2a and IgG2b in culture supernatants of spleen cells were dose-dependently increased by in vivo treatment of ginseng extract, suggesting that both Th1- and Th2-type cytokines were involved in IgG production. Our observation in this study demonstrated that the Chinese herbal drug ginseng was able to regulate antibody production by augmenting Th1- (IL-2, IFN-γ) and Th2-type (IL-4, IL-10) cytokine production.

Keywords: Ginseng; Extract Preparation; Immunoglobulin; Cytokines; Mice.

Introduction

Ginseng (Panax ginseng, C.A. Meyer) has been a popular herbal remedy used in eastern Asian cultures for thousands of years. Based on its major pharmacological effects, Panax ginseng is used in traditional Chinese medicine to enhance stamina and capacity to cope with fatigue and physical stress. The mechanisms of ginseng actions remain unclear, although there is an extensive literature that deals with the effects of ginseng on a variety of diseases as well as various infections (Awang, 1999; Ernst, 2002). Panax ginseng has also been shown to accelerate hepatic lipogenesis and increase glycogen storage, which could contribute to an antidiabetic effect (Yokozawa et al., 1975). Persons who consumed fresh ginseng did show a significantly reduced risk ratio for cancer (Yun and Choi, 1998). Furthermore, ginseng has been proposed to be a potent immunomodulator. Ginseng enhanced production of macrophages, B- and T-cells, natural killer (NK) cells and colonic activity of bone marrow (Klein et al., 2000). The ethanol-insoluble fraction of an aqueous extract of ginseng was found to induce proliferation of splenocytes and generate activated killer cells in vitro (Yun et al., 1993). A systematic evaluation of multiple factors in the immune system revealed that Panax ginseng stimulated basal NK cell activity and promoted a recovery of NK function from cyclophosphamide-induced immunosuppression in mice (Kim et al., 1990). In addition, the extract of Ginseng radix was demonstrated to enhance the anti-Candida activity of macrophages in vitro and to prolong the survival time of Candida albicans-infected C3H/HeJ mice (Akagawa et al., 1996).

An acidic polysaccharide from ginseng named ginsan induced the generation of CD8+ LAK cells from both NK and T cells. The same report also demonstrated that ginsan induced the expression of mRNA for interleukin (IL)-2, interferon (IFN)-γ, IL-1α and granulocyte macrophage colony stimulating factor (GM-CSF) in C57BL/6 mouse spleen cells when the cells were cultured in the medium containing concanavalin A (Con A) plus ginsan (Kim et al., 1998). Recently, the anti-inflammatory effects of several ginsenosides derived from Panax ginseng were studied. These ginsenosides suppressed expression of cyclooxygenase-2 and activation of NF-kappaB in mice (Surh et al., 2002).

The previous documents suggested that ginseng showed profound effects on the humoral immune response. Oral administration of the aqueous extract of ginseng for 5 to 6 days significantly increased the primary immunoglobulin (Ig)M response by 50%, and augmented the secondary IgG and IgM response by 50% and 100%, respectively (Jie et al., 1984). The
volunteers receiving oral administration of a standardized extract of ginseng root, Ginsana G115, induced a higher immune response in vaccination against influenza in comparison with the individuals receiving placebo (Scaglione et al., 1996). However, in an athymic rat model, subcutaneous administration with ginseng extracts after rats were challenged with Pseudomonas aeruginosa reduced anti- P. aeruginosa IgM and IgA antibody levels (Song et al., 1997a). In a rat model of chronic P. aeruginosa pneumonia, subcutaneous injection of an aqueous extract of ginseng resulted in an increase in IgG2a level but a decrease in IgG1 level in comparison with that in the control group. The authors suggested that the change from IgG1 to IgG2a subclasses indicated a possible shift from T-helper (Th)2- to Th1-type immune response (Song et al., 1998). Long-term oral administration of ginseng extract decreased the serum level of γ-globulin by 56%. Among the immunoglobulin isotypes, serum IgG1 was dose-dependently decreased to 68% of control value (Kim et al., 1997). A recent study in lactating cows indicated that the ginsenoside R (b1) significantly augmented antibody production and lymphocyte proliferation in response to pokeweed mitogen (PWM), Con A and Staphylococcus aureus antigens (Hu et al., 2003).

In this study, the specific pathogen-free mice were intraperitoneally injected with 50% ethanol extract of ginseng for 3 consecutive days. Both the immunoglobulin (IgG, IgA, IgM) and cytokine (IL-2, IFN-γ, IL-4, IL-10) production by the spleen cells were measured after the treatment. The relatively short-term effect of ginseng extract was estimated, and the possible relation between cytokine production and immunostimulating effects of ginseng was discussed.

Materials and Methods

Preparation of Herbal Drug Extract

A batch of herbal medications was purchased from the Lao-Chen-Ge Chinese herbal drugstore, De-Hua Street, Taipei. The herbs were ground into dried powder. The ginseng (5 g) powder was mixed and suspended in 100 ml of 50% ethanol. The drug suspension was boiled until half the volume of liquid remained. The suspension was then spun at 10,000 g for 30 minutes and the supernatant was collected and dried with a Speed Vac. The dried extracts were reconstituted using sterile phosphate buffered saline (PBS) to make a stock of 100 mg drug/ml and sterilized with a 0.2 µm Millipore filter before used.

Animal Treatment and Cell Culture

For the drug treatment, the animals were divided into four groups and injected intraperitoneally (i.p.) with 0.5 ml of ginseng extract, ranging from 0.4, 2 to 4 g/kg body weight daily (g/kg/day) for three days. The control group was composed of mice injected with an equal volume of PBS. Mice were sacrificed at day 4. The serum from individual mice was collected and the spleen cells isolated. The spleen cells (5 × 10^5 cells/ml) were cultured in a medium containing RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, antibiotics and lipopolysaccharide (LPS; 1 µg/10^5 cells) for 5 days. The immunoglobulin concentrations
in the culture supernatants were estimated using the enzyme-linked immunosorbent assay (ELISA) technique. To investigate the effects of ginseng on cytokine production, the mice were also treated as described, but the spleen cells were cultured in the presence of 1 µg/ml Con A for 3 days. Culture supernatants of spleen cell were collected, and the cytokine concentrations were measured using the ELISA technique.

ELISA

IgA, IgG, IgM, IgG1, IgG2a and IgG2b concentrations were measured using a sandwich ELISA technique. The capture antibody for the assay was a rabbit anti-mouse IgG + IgA + IgM antibody (Zymed Laboratory, CA). The secondary antibody for the assay was the horseradish peroxidase (HRP)-conjugated goat anti-mouse IgA (1:500 diluted; Zymed Laboratory, CA), goat anti-mouse IgG (1:10,000 diluted; whole IgG molecule; Jackson ImmunoResearch, PA), and goat anti-mouse IgM (1:2000 diluted; heavy chain-specific; Jackson ImmunoResearch, PA). The HRP-conjugated goat anti-mouse IgG1, IgG2a and IgG2b (Zymed Laboratory, CA) were diluted to 1:1000. Briefly, a 96-well microtiter plate (Nunc-Immuno Plate, MaxiSorp, Nunc, Denmark) was precoated with 100 ng/well of capture antibodies at 4°C overnight. The plate was washed with PBS–0.05% Tween 20 solution and blocked with PBS-1% gelatin. After the blocking, the properly diluted samples and standard were added (100 µl/well). Standards for IgG, and IgA had a range from 0.2 to 0.0031 µg/ml, standards for IgM had a range from 1 to 0.0312 µg/ml, and standards for IgG1, IgG2a and IgG2b had a range from 0.1 to 0.0016 µg/ml. The plate was then incubated at 37°C for 2 hours. At the end of incubation, a HRP-conjugated secondary antibody was added (100 µl/well). After 1 hour of incubation at 37°C, the color was developed using a substrate solution containing 0.1 M citrate buffer, pH 4.5, 0.03% H₂O₂ and 0.1% of o-phenylenediamine. The absorbance at 490 nm in each well was read using an ELISA reader (EL311, BioTek, VT), and the data was analyzed using log-logit model.

For the quantitative analysis of IL-2, IFN-γ, IL-4 and IL-10, cytokine ELISA set purchased from R&D Systems (MN, USA) was used. The capture antibody was a rat monoclonal antibody to mouse cytokine and the detection antibody was a biotinylated goat anti-cytokine polyclonal antibody. The color was developed by incubating the plate with an HRP-conjugated streptavidin (Zymed, CA, USA), followed by a substrate solution containing hydrogen peroxide and tetramethylbenzidine (TMB). The reaction continued for 30 minutes at room temperature, and was stopped by adding 100 µl of 2N of sulfuric acid. The absorbance at 450 nm in each well was read using an ELISA reader (EL311, BioTek, Winooski, VT), and the data was analyzed using a log-logit model.

Synthesis of cDNA

The level of Th1 type (IL-2, IFN-γ) and Th2 type (IL-4, IL-10) mRNA in the spleen cells was estimated using reverse transcriptase-polymerase chain reaction (RT-PCR) analysis.
36 hours after culture in the presence of 1 µg/ml of Con A. Spleen cells (1 × 10^7 cells) isolated from ginseng-treated animals were washed twice with 1× RNase-free PBS. The cell pellet was mixed with 1 ml of TRIzol reagent (GIBCO-BRL, MD), and the mixture was forced to pass through a 25G needle five times to release RNA from the cells. This homogenate was then vigorously mixed with 0.2 ml chloroform. After sitting at room temperature for 10 minutes, the mixture was spun at 4°C for 15 minutes to separate the organic and aqueous layers. The aqueous layers were removed into a new tube and RNA was precipitated with 0.5 ml of isopropanol. The precipitate was then resuspended in 30 µl of RNase-free water, and a 5 µl aliquot was removed for RNA quantification using GeneQuant II (Pharmacia Biotech, Piscataway, NJ). RNA in RNase-free water (2 µg in 10 µl) was mixed with 2.5 µl of oligo (dT)₁₅ solution (40 pM; Promega, WI). The solution was heated at 70°C for 10 minutes, followed by cooling at room temperature for 10 minutes and then transferred onto ice. A reaction mixture containing 4 dNTPs (Boehringer Mannheim, Germany), DTT (GIBCO/BRL, MD), reverse transcriptase (M-MLV; Promega, WI) and RNasin (Promega, WI) was subsequently mixed with RNA. The reaction was carried out at 37°C for 60 minutes to synthesize the cDNA.

**PCR**

cDNA (5 µl) was mixed with 0.5 µl 4 dNTP, 10 µl primer mix (2.5 µM each), 0.5 µl Taq polymerase, 2 µl MgCl₂ (2 mM), and 5 µl PCR buffer. The DEPC-treated water was then added to make a volume up to 50 µl in total. The primers (Promega, WI) used for PCR amplification were as follows: IL-10 sense primer: 5′-ATGCAG GACTTTAAGGGTTACTTG-3′; IL-10 antisense primer: 5′-TAGACACCTTGGT CTTGGAGCTTAA-3′; IL-4 sense primer 5′-TGCCCTCCAAGAACACAACGT-3′; IL-4 antisense primer: 5′-AACGTAAGCTTGCTGTTGCTT-3′; IL-2 sense primer: 5′-GACACTTTGTGCTTTGCTCA-3′; IL-2 antisense primer: 5′-TCAATTCTGTT GCTCTTG TG-3′; IFN-γ sense primer: 5′-GCAGAGCCTAATCTCCTCCT-3′; IFN-γ antisense primer: 5′-ATGCTCCTCAGCCTGAAAC-3′; β₂ microglobulin (internal control) sense primer: 5′-TGACCGCTTGTGATGCTATC-3′; β₂ microglobulin antisense primer: 5′-CAGTGTGACGCGAGGTATAG-3′. The PCR conditions were denaturation at 94°C for 50 seconds, annealing at 60°C for 45 seconds and primer extension at 72°C for 45 seconds. After 35 cycles (40 cycles for IL-10) of amplification, the PCR products were subjected to gel electrophoresis through 1.5% agarose (Sigma, MO) containing ethidium bromide at 80 V. The amplicons were visualized under UV light.

**Statistical Analysis**

Data from the control or drug-treatment groups were tested by ANOVA. The difference between the two means was assessed using the Student’s t-test. Probability values of < 0.05 were considered to be significant.
Results

Ginseng Induced an Increase in Immunoglobulin Production

Both the sera and spleen cells isolated from the mice injected with various doses of ginseng or the same volume of PBS (control group) were collected. The spleen cells were cultured in vitro for 5 days with the presence of 1 µg/ml LPS. The amount of immunoglobulins (IgG, IgA and IgM) in sera and culture supernatants were measured using ELISA. The results indicated that the serum levels of IgM, IgG and IgA were significantly elevated after the mice were i.p. injected with 4 g/kg/day of 50% ethanol-extract of ginseng for 3 consecutive days (Fig. 1). The IgM level was evenly elevated at the relatively low dose (2 g/kg/day). Under in vitro condition, the LPS-stimulated spleen cells showed a dose-dependent increase in secretion of IgM and IgG (Fig. 2). For the spleen cells isolated from the mice treated with 0.4 and 2 g/kg/day of ginseng extract, the amount of IgA in culture supernatant was also increased. However, at a higher dosage (4 g/kg/day), the amount of IgA secretion began to decline but was still significantly higher than that of the control group.

The predominant immunoglobulin in serum was IgG, which had a range between 10 to 20 mg/ml (Fig. 1). The serum level of IgM was relatively low, which was only 0.1 to 0.2 mg/ml. However, the predominant immunoglobulin secreted by the LPS-stimulated spleen cells was IgM, which was between 0.5 to 1.5 µg/ml in culture supernatant (Fig. 2). Both the IgG and IgA secretion were less than 80 ng/ml. This simply demonstrated that LPS was a T-independent antigen. The LPS-activated B-lymphocytes predominantly produced IgM and showed no class switching. Therefore, the ginseng-induced increase in immunoglobulin secretion might be due to either the class-switching by ethanol-extract of ginseng being induced or the secretion of the pre-existing immunoglobulin producers being augmented.

Ginseng Induced an Increase in Cytokine Production

In order to study the possible role of cytokines in ginseng-induced increase of immunoglobulin production, both the sera and spleen cells isolated from the ginseng-treated mice were assayed for cytokine level and secretion, respectively. The results indicated that the serum level of IL-2, IFN-γ (Th1-type cytokines) was significantly elevated after the mice were i.p. injected with 2 g/kg/day or higher dose of 50% ethanol-extracted of ginseng for 3 consecutive days. The serum concentration of IL-2 increased five to ten folds, and that of IFN-γ also increased about ten to 15 folds (Fig. 3). The serum level of IL-4 and IL-10 (Th2-type cytokines) were also elevated by the ginseng treatment. Ginseng treatment dose-dependently increased the serum level of IL-10. The mice injected with 2 g/kg/day or more of ginseng extract also showed a significant increase in the serum level of IL-4. However, the serum level of IL-4 was in a range between 0.1 to 3 pg/ml, which was approximately 100-fold lower than that of IL-10 (Fig. 4). For the spleen cells isolated from ginseng-treated mice, the concentrations of Th1- and Th2-type cytokines in culture supernatants were also increased significantly in comparison to that of the control group (Figs. 5 and 6). However, the amount of Th1-type cytokines produced by spleen cells was approximately two-fold higher than that of Th2-type cytokines.
GINSENG ENHANCES IMMUNOGLOBULIN AND CYTOKINE PRODUCTION

Figure 1. The level of immunoglobulin in sera collected from ginseng-treated mice. BALB/c mice were i.p. injected with various doses of ginseng, ranging from 0.4 to 4 g/kg/day, for 3 consecutive days. The control group consisted of mice injected with an equal volume of PBS. Sera were collected and assayed for IgG, IgA and IgM concentrations using ELISA. Data were mean ± SEM of six similar experiments. *p < 0.05 from the control group.

Figure 2. Ginseng induced an increase in immunoglobulin secretion by spleen cells in vitro. BALB/c mice were i.p. injected with various doses of ginseng, ranging from 0.4 to 4 g/kg/day, for 3 consecutive days. The control group consisted of mice injected with an equal volume of PBS. Spleen cells were isolated after the treatment and then incubated for 5 days with 1 µg/ml of LPS. Supernatants were harvested and assayed for Ig concentrations using ELISA. Data were mean ± SEM of six similar experiments. *p < 0.05 from the control group.
Figure 3. The level of Th1-type cytokines in sera collected from ginseng-treated mice. BALB/c mice were i.p. injected with various doses of ginseng, ranging from 0.4 to 4 g/kg/day, for 3 consecutive days. The control group consisted of mice injected with an equal volume of PBS. The sera were collected and assayed for IL-2 and IFN-γ concentrations using ELISA. Data were mean ± SEM of six similar experiments. *p < 0.05 from the control group.

Figure 4. The level of Th2-type cytokines in sera collected from ginseng-treated mice. BALB/c mice were i.p. injected with various doses of ginseng, ranging from 0.4 to 4 g/kg/day, for 3 consecutive days. The control group consisted of mice injected with an equal volume of PBS. The sera were collected and assayed for IL-4 and IL-10 concentrations using ELISA. Data were mean ± SEM of six similar experiments. *p < 0.05 from the control group.
GINSENG ENHANCES IMMUNOGLOBULIN AND CYTOKINE PRODUCTION

Figure 5. Ginseng induced an increase in Th1-type cytokine secretion by spleen cells in vitro. BALB/c mice were i.p. injected with various doses of ginseng, ranging from 0.4 to 4 g/kg/day, for 3 consecutive days. The control group consisted of mice injected with an equal volume of PBS. Spleen cells were isolated and in vitro cultured for 72 hours in the presence of 1 µg/ml of Con A. Supernatants were harvested and assayed for Th1-type cytokine (IL-2 and IFN-γ) by ELISA. Data were mean ± SEM of six similar experiments. *p < 0.05 from the control group.

Figure 6. Ginseng induced an increase in Th2-type cytokine secretion by spleen cells in vitro. BALB/c mice were i.p. injected with various doses of ginseng, ranging from 0.4 to 4 g/kg/day, for 3 consecutive days. The control group consisted of mice injected with an equal volume of PBS. Spleen cells were isolated and in vitro cultured for 72 hours in the presence of 1 µg/ml of Con A. Supernatants were harvested and assayed for Th2-type cytokine (IL-4 and IL-10) by ELISA. Data were mean ± SEM of six similar experiments. *p < 0.05 from the control group.
Ginseng Augmented Expression of Th1- and Th2-Type Cytokine mRNA

To further confirm the results from ELISA, the spleen cells were isolated after the treatment and cultured for 36 hours in the presence of 1 µg/ml of Con A. Total mRNA was isolated and assayed for mRNA expression using RT-PCR. Results of RT-PCR correlated well with that of ELISA in which expression of IL-2 and IFN-γ mRNA were dose-dependently enhanced by ethanol extract ginseng (Fig. 7). The levels of IL-4 and IL-10 mRNA expression were also elevated in the spleen cells from ginseng-treated mice in comparison to that of the control group (Fig. 7).

Ginseng Extract Increased Secretion of IgG Subtypes by Spleen Cells

To demonstrate that Th1- and Th2-type cytokines were involved in IgG production, the amount of IgG1, IgG2a and IgG2b secretion by spleen cells in vitro were assayed. Results indicated that the concentrations of IgG1 and IgG2b in culture supernatants of spleen cells were dose-dependently increased by in vivo treatment of ginseng extract (Fig. 8). An increase in IL-4 production resulted in class-switching from IgM producer to IgG1 and IgG2b producer. Therefore, it further confirmed that IL-4 production was enhanced by ginseng extract. The amount of IgG2a secretion was significantly increased after the mice were treated with ginseng extract at as low as 0.4 g/kg/day. Since IFN-γ was a potential inducer for class-switching from IgM to IgG2a, this result also confirmed that ginseng-treatment did enhance IFN-γ production.

Figure 7. Detection of cytokine mRNA expression in spleen cells isolated from ginseng-treated mice. BALB/c mice were i.p. injected with various doses of ginseng, ranging from 0.4 to 4 g/kg/day, for 3 consecutive days. The control group consisted of mice injected with an equal volume of PBS. Spleen cells were isolated and in vitro cultured for 36 hours in the presence of 1 µg/ml of Con A. The total mRNA was isolated from the spleen cells (1 × 10^7 cells), cDNA was synthesized and then amplified using PCR. Lane 1: control, Lane 2: 0.4 g/kg/day, Lane 3: 2 g/kg/day, and Lane 4: 4 g/kg/day.
Discussion

Our observations from this study demonstrated that the Chinese herbal drug ginseng was able to regulate antibody production by augmenting Th1- (IL-2, IFN-γ) and Th2-type (IL-4, IL-10) cytokine production. A significant increase in level of cytokine mRNA in the spleen cells isolated from ginseng-treated mice suggested that the ingredients in ginseng extract either induced the gene expression of cytokines or increased the stability of the cytokine mRNA during the period of ginseng treatment. The cytokine secretion was subsequently increased in secondary lymphoid tissues (e.g. spleen). Th1- (IL-2, IFN-γ) and Th2-type (IL-4, IL-10) cytokines produced by spleen cells then induced an increase in IgM, IgG and IgA production by either directly stimulating the pre-existing Ig producers or inducing the class-switching from IgM producers to IgG and IgA producers. The increase in immunoglobulin production resulted in elevation of the serum levels of IgM, IgG and IgA. Data from in vitro culture of the spleen cells, either stimulated by Con A or LPS, confirmed the potential of ginseng extract to augment both cytokine and immunoglobulin production.

The experimental design of the present study was fairly different from that of previous reports in terms of preparation of the ginseng extract, dosage and protocol of the treatment. In the study reported by Jie et al. (1984), an aqueous extract of ginseng was prepared by boiling crushed ginseng roots in water. The extract was administrated orally to mice for 5 to 6 days. The daily doses of ginseng extract were 0.01, 0.05 and 0.25 g/kg/day. At the highest

Figure 8. Ginseng induced an increase in IgG subclasses secretion by spleen cells in vitro. BALB/c mice were i.p. injected with various doses of ginseng, ranging from 0.4 to 4 g/kg/day, for 3 consecutive days. The control group consisted of mice injected with an equal volume of PBS. Spleen cells were isolated after the treatment and then incubated for 5 days with 1 µg/ml of LPS. Supernatants were harvested and assayed for IgG subclasses (IgG1, IgG2a and IgG2b) concentrations using ELISA. Data were mean ± SEM of six similar experiments. *p < 0.05 from the control group.
dose, i.e. 0.25 g/kg/day, both the primary IgM response and the secondary IgG and IgM responses were increased by 50%. The author also observed an enhancement of interferon production in non-stimulated spleen cells. We had similar findings, but the extract of ginseng was prepared by boiling the ground ginseng roots in 50% ethanol, instead. The extract was administered i.p. for 3 days. The daily doses of ginseng extract were 0.4, 2 and 4 g/kg/day, which was significantly higher than that of Jie’s study. However, we observed an increase in secretion of immunoglobulin at the lowest dose (0.4 g/kg/day), which was only slightly higher than the dose used by Jie’s group.

However, Song’s group showed different results. In an athymic rat model, they subcutaneously administrated the rats with an aqueous extract of ginseng after the animals were challenged with *Pseudomonas aeruginosa*. The daily dosage of the ginseng extract was 0.025 g/kg/day and the rats were treated with the extract for 10 days. After the treatment, they observed a reduction in serum levels of anti-*P. aeruginosa* IgM and IgA antibodies (Song et al., 1997b). The extract used by Song’s group was water-soluble instead of the 50% ethanol-soluble fraction of ginseng. The dose used in Song’s study was relatively low and the period of treatment in their study was longer than that in our study. In addition, they monitored the effect of ginseng on the antigen-specific antibody response instead of on polyclonal antibody production.

In a rat model of chronic *P. aeruginosa* pneumonia, subcutaneous injection of an aqueous extract of ginseng at a dose of 0.025 g/kg/day for 2 weeks resulted in an increase in IgG2a level but a decrease in IgG1 level in comparison to that of the control group (Song et al., 1998). The authors suggested that the change from IgG1 to IgG2a subclasses indicated a possible shift from Th2- to Th1-type immune response. Kim’s group studied the long-term oral administration of ginseng extract to healthy female mice. They found that oral administration of ginseng at doses of 0.03 and 0.15 g/kg/day for 52 days decreased the serum level of γ-globulin by 56%. However, the Ig isotypes, including IgG2a, IgG2b, IgG3, IgM and IgA, in serum was unchanged, but serum IgG1 was dose-dependently decreased to 68% of the control value (Kim et al., 1997). Namely, the healthy condition and the length of administration might result in an opposite effect on the production of Ig isotypes. Kim’s group previously studied ginsan, an acidic polysaccharide from ginseng. When the spleen cells were cultured *in vitro* with the presence of ginsan, they found that ginsan induced the generation of CD8+ LAK cells from both NK and T cells. The same report also demonstrated that ginsan induced the expression of mRNA for IL-2, IFN-γ, IL-1α and GM-CSF in C57BL/6 mouse spleen cells when the cells were cultured with Con A plus ginsan (Kim et al., 1997). In our study, we were unable to find any preference between Th1- and Th2-type immune responses after the mice were i.p. injected with 50% ethanol extract of ginseng.

Although ginseng has been used in Eastern Asian countries for more than a thousand years, the biological activity, pharmacological effect and its active ingredients remain to be determined. Evidence from controlled clinical trials did not support the use of ginseng to treat its established indications (Ernst, 2002). Furthermore, the way to prepare ginseng extract, the dosage, the route of administration, and the protocol of treatment can indeed affect the efficacy of ginseng. Therefore, more studies are needed to establish the critical parameters.
Acknowledgments

This work was presented by Chian-Jiun Liou to the Department of Biology, National Taiwan Normal University in partial fulfillment of the requirement for a PhD degree.

References


