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Immunomodulatory Effect of Acidic Polysaccharide Fraction from Korean Red Ginseng (*Panax ginseng*)

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Abstract – Effects of red ginseng acidic polysaccharides (RGAP) on immune system were studied. The proliferation of spleen cells was induced by RGAP treatment *per se*. Cotreatment of lipopolysaccharide (100 μg/ml) or concanavalin A (1 μg/ml) with RGAP further stimulated the spleen cell proliferation. BALB/c mice treated with RGAP showed a slight splenic hyperplasia and increased antibody forming cell response to sheep red blood cells. Flow cytometry analysis revealed an influx of macrophages in the mice treated with RGAP.

Key words – *Panax ginseng*, red ginseng, acidic polysaccharide, antibody forming cell, macrophage

Introduction

The root of *Panax ginseng* C. A. Meyer is one of traditional and folk medicines which have been used for many therapeutic purposes in the orient. Immunomodulatory activity is one of major pharmacological effects of ginseng. Polysaccharides isolated from ginseng root was found to show mitogenic activities (Eun *et al.*, 1989), hypoglycemic activities (Konno *et al.*, 1983; Konno *et al.*, 1985) and antitumor activities (Moon *et al.*, 1983; Lee *et al.*, 1997). It was also reported that acidic polysaccharide was more effective than neutral polysaccharide in the immunostimulating activities (Kim *et al.*, 1990).

While extensive studies on the immunomodulating activities of ginseng root were performed, its effector mechanism is not clear. This study demonstrates that splenic influx of macrophages might be involved in the increased antibody response to sheep red blood cells in the mice treated with acidic polysaccharide from red ginseng.

Materials and Methods

Mice – Specific pathogen-free female BALB/c mice were obtained from the Animal Breeding Laboratory at Korea Research Institute of Chemical Technology. The mice were received at 4-5 weeks of age and acclimated for at least 1 week. All animals were maintained on gamma-irradiated Jeil Lab Chow (Taejon, Korea) and ultraviolet-irradiated tap water *ad libitum*. Mice at the age of 6-8 weeks were used in this study. The animal quarters were strictly maintained at 23±3°C and 40-60% relative humidity. A 12 hour light/dark cycle was used with an intensity of 150-300 lux.

Reagents – Concanavalin A (Con A), lipopolysaccharide (LPS), DEAE-dextran, carbazole, glucose, glucuronic acid, bovine serum albumin (BSA), 2-mercaptoethanol (2-ME) and dialysis tube were supplied from Sigma Chemical Co. (St. Louis, MO). Absolute alcohol and sulfuric acid were from Fluka (Buchs, Switzerland). Earle's balanced salt solution (EBSS), HEPES, fetal bovine serum (FBS), L-glutamine, penicillin/streptomycin solution, RPMI 1640 medium, agar and guinea pig complement were from GIBCO (Grand Island, NY). Sheep red blood cells (SRBC) were from Cedarlane (Ontario, Canada). Korean red ginseng was from Korea Ginseng Corporation (Taejon, Korea). Cell Titer 96 Aq_{ueous} Non-Radioactive Cell Proliferation Assay System was from Promega (Madison, WI). The other reagents used were of extra pure grades.

Preparation of red ginseng acidic polysaccharide (RGAP) – Korean red ginseng was cut to mill. Powdered red ginseng was percolated with 5 volumes of 85% ethanol to extract off ethanol-soluble materials. Remaining residues were then percolated

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with 5 volumes of distilled water and the resulting water-soluble fractions were concentrated with a vacuum evaporator. The concentrates were dialyzed against running tap water for 7 days to completely cut off small molecules less than 15 kDa. Aggregated insolubles were centrifuged off and the supernatants were then precipitated with 4 volumes of absolute ethanol. The precipitate was dried in a vacuum drying oven and used as red ginseng acidic polysaccharide (RGAP).

Determination of lymphocyte proliferation – Spleen was aseptically removed from BALB/c mouse sacrificed by cervical dislocation. Using a flat end of sterile syringe plunger, spleen was gently crushed in a ice-cold EBSS. Cell suspension was placed for 10 min to precipitate the unbroken cell debris. Upper part of cell suspension was carefully transferred to a new centrifugal tube and centrifuged at 400×g for 10 min. Cell pellet was resuspended in RPMI 1640 culture medium containing 10% FBS, 15 mM HEPES, 5×10^{-2} mM 2-ME, 2 mM glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Spleen cells at 2×10^5 cells/well were cultured in a 96 well plate at 37 under humidified 5% CO_2-95% air incubator. At 72 hours after culture, cell proliferation was assayed using Promega Cell Titer 96 Aq_{ueous} Non-Radioactive Cell Proliferation Assay Systems following the instruction manual provided.

Assay of *in vivo* antibody forming response (AFC) to sheep red blood cells (SRBCs) – Specific pathogen-free BALB/c mice were injected intraperitoneally with sterilized RGAP solution for consecutive seven days. Control mice were injected with a sterilized saline solution. Four days before the assay, mice were sensitized with 5×10^8 SRBC in 0.5 ml of EBSS intraperitoneally. Single cell suspensions of splenocytes were prepared in 3 ml EBSS, washed and resuspended in 3 ml EBSS. Spleen cells were then diluted 30-fold by resuspending a 100 μl aliquot of each cell suspension in 2.9 ml of EBSS. The number of AFCs was determined using a modified Jern Plaque assay (Holsapple, 1995). Briefly, 0.05% DEAE-dextran was added into melted 0.5% agar in EBSS and maintained at 47°C throughout the assay. Then 400 μl of melted agar was dispensed into 12×75 mm heated glass tubes (Corning, NY), followed by the addition of 25 μl of SRBC, 50 μl of spleen cell suspension and 25 μl of guinea-pig complement. The SRBC were washed at least three times with EBSS before use. A 200 μl aliquot from the tube was then immediately pipetted on to a 100×15 mm petri dish and the agar solution was covered with 24×40 mm microscopic cover glass. The petri dishes were placed at room temperature for several minutes to allow the agar to solidify and were then incubated at 37°C for 3 h to form haemolytic plaques in a humidified 37°C incubator. Following the incubation, the AFCs were then counted at 6.5 × magnification using a Bellco plaque viewer (Bellco Biotechnology, NJ). The cell number of each spleen was determined using a coulter counter. The results were expressed as AFCs/spleen or AFCs/10^6 spleen cells.

Flow cytometric analysis of splenic macrophages – Single cell suspensions of splenocytes were prepared in 3 ml EBSS, washed and resuspended in 3 ml EBSS. Spleen cells were hemolyzed by mixing 500 μl of spleen cell suspensions with 9.5 ml of lysis buffer (0.017 M Tris, 0.75% NH_4Cl. pH 7.2) for 10 min at room temperature and centrifuged off the supernatant. Resulting pellet was suspended in 0.5 ml of PBSN-BSA (PBS containing 0.1% sodium azide and 1% BSA) and then cell numbers were counted. Appropriate number of cells (2×10^6 cells/100 μl) was mixed with same volume of antibody solutions (0.5 μg/100 μl in PBSN-BSA) and incubated at 4°C for 30 min in the dark. After incubation, unbound antibodies were removed by centrifugation and a subsequent washing with PBSN-BSA without antibodies was performed. The resultant pellet was resuspended in 0.5 ml of PBSN-BSA and analyzed by Facsort (Becton-Dickinson, USA).

Chemical analysis of RGAP – Neutral sugar and acidic sugar were determined by the method of Dimler (Dimler *et al.*, 1952) and Galambis (Chaplin *et al.*, 1987) using glucose and glucuronic acid as respective standards. Protein was determined by Lowry method (Lowry *et al.*, 1951) using BSA as a standard.

Statistical analysis – Statistical significance was determined by Student's t-test.

Results and Discussion

In vitro polyclonal cell proliferation and *in vivo* haemolytic AFC assays are effective tools to assess the functional status of immune system. We used these assays to evaluate the effects of RGAP on immune system. RGAP from ethanol-insoluble and water-soluble fraction of red ginseng was composed

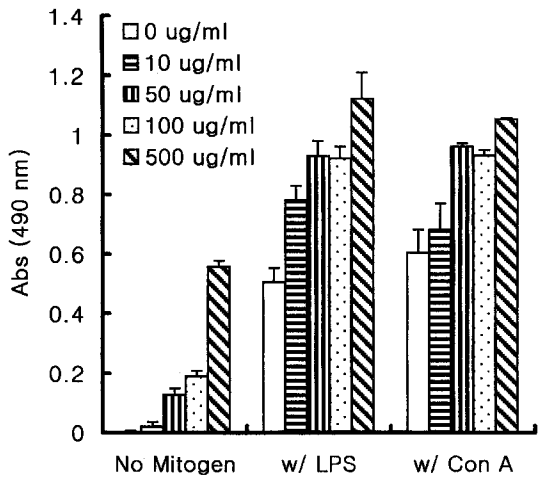

Fig. 1. Effects of RGAP on spleen cell proliferations. Spleen cells (2×10^5/well) were cultured with RGAP alone (No Mitogen), or RGAP simutaneously with LPS (100 μg/ml) or Con A (1 μg/ml) for 72 hr at 37°C CO_2 incubator. Each value represents mean±S.D. of triplicates. All points are significantly different from control at $p<0.05$.

Table 1. Chemical composition of RGAP (w/w, %)

Neutral sugar[1)]	Acidic sugar[1)]	Protein[1)]
28.3%	56.9%	< 0.1%

of 56.9% acidic sugar and 28.3% neutral sugar (Table 1). Protein was scarcely detected, not more than 0.1%.

Spleen cells were induced to proliferate by the addition of RGAP (Fig. 1). Cotreatment of LPS (100 μg/ml) or Con A (1 μg/ml) with RGAP further stimulated spleen cell proliferations dose-dependently. The spleen cell proliferation induced by 500 μg/ml of RGAP was comparatively equal to that of LPS (100 μg/ml).

RGAP treatment in mice induced increases of spleen weights and spleen cell numbers (Fig. 2a & 2b). The numbers of AFCs/10^6 spleen cells and AFCs/spleen were also increased in the mice treated with RGAP (Fig. 2c & 2d). In a parallel with increased AFC responses, the percentile and number

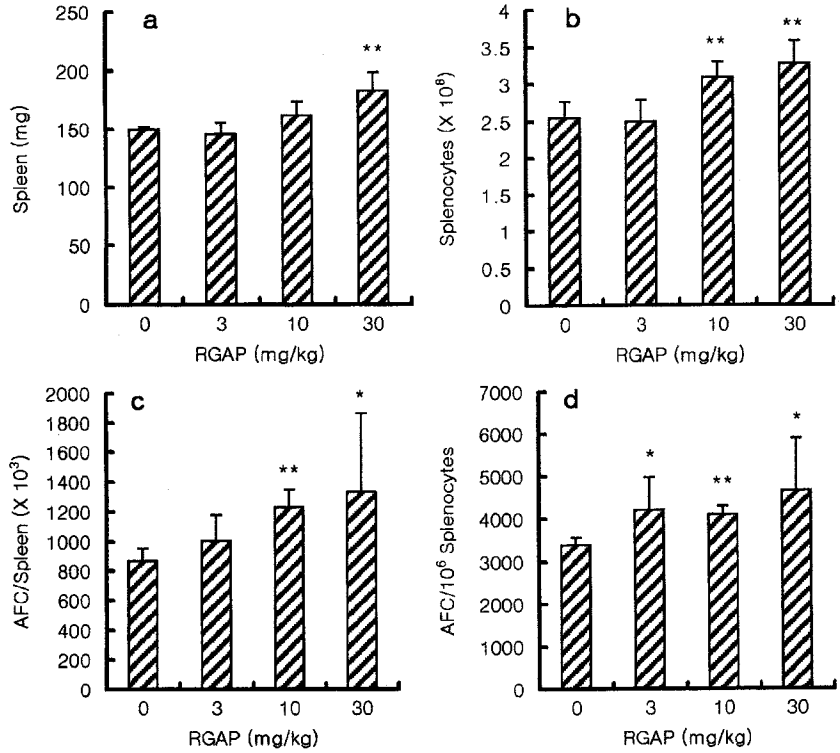

Fig. 2. Effects of RGAP on *in vivo* AFC response to SRBCs. RGAP was administered intraperitoneally for consecutive seven days. Zero denotes control mice treated with saline solution. Spleen weight (a), spleen cell numbers (b), AFC/Spleen (c) and AFC/10^6 splenocytes (d) were presented. Each value represents mean±S.D. of 8 mice. * and ** denote significant difference from control at $P<0.05$ and $P<0.01$, respectively.

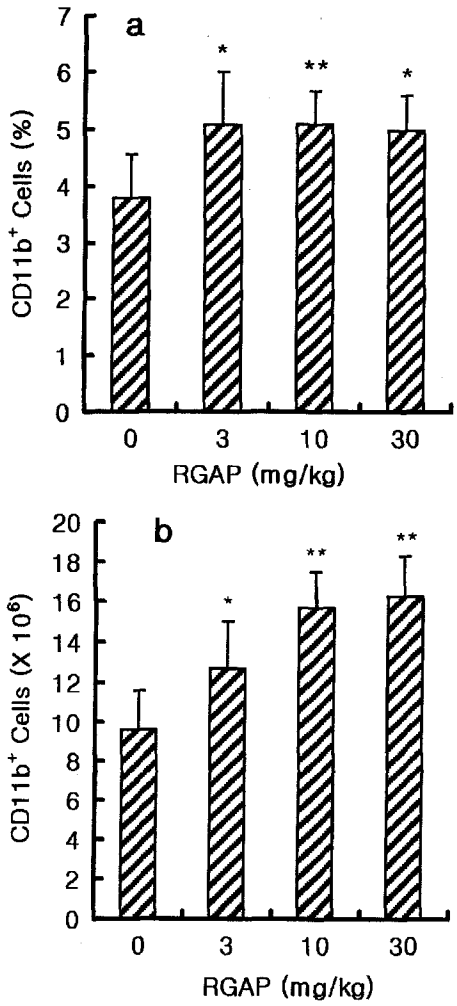

Fig. 3. Effects of RGAP on splenic macrophages. RGAP was administered intraperitoneally for consecutive seven days. The proportion (a) and number (b) of $CD11b^+$ cells are presented. Each value represents mean±S.D. of 8 mice. * and ** denote significant difference from control at $P<0.05$ and $P<0.01$, respectively.

of $CD11b^+$ cells including macrophages were significantly increased in the mice treated with RGAP (Fig. 3a & 3b). Fractions of $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD45/B220^+$ cells were not changed by the treatment of RGAP (data not shown).

It is known that a splenic influx of macrophages is responsible for the enlargement of spleen. Splenomagaly was observed in the immunization of *Haemophilus influenza* simultaneously with an increased number of splenic macrophages (Ambrosino *et al.*, 1992). It was also known that a major cytokine of macrophages, interleukin 1 contributed to granulocytic hyperplasia of spleen (Benjamin et al., 1989). Taken together, it is assumed that the increase of macrophages is responsible for the gain of spleen weight in the mice treated with RGAP.

This study demonstrates the splenic increase of macrophages might be the primary cause of immunomodulating activities of RGAP in mice. Further studies on the effects of macrophages in the RGAP-treated mice are in progress.

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