*Arch. Pharm. Res.* **13**(4), 330-337 (1990)

# Study on Antitumor and Immunomodulating Activities of Polysaccharide Fractions from *Panax ginseng*: Comparison of effects of neutral and acidic polysaccharide fraction

**Young Sook Kim*, Kyu Sang Kang and Shin Il Kim**
*Korea Ginseng and Tobacco Research Institute, Taejon 305-345, Korea*
*(Received September 25, 1990)*

**Abstract**□The crude polysaccharide from *Panax ginseng* prepared by hot water extraction and precipitation with ethanol was further fractionated into neutral and acidic fractions by DEAE-cellulose ion exchange chromatography. The chemical compositions were 85.0% carbohydrate and 15.0% protein for the neutral fraction, and 28.4% carbohydrate, 10.0% protein and 29.0% uronic acid for the acidic fraction. The acidic fraction was more effective in increasing of the ratio of spleen to body weight, the number of antibody secreting cells to SRBC and phagocytic activity of reticuloendothelial system, as well as antitumor activity against the solid form of sarcoma 180 in ICR mice than the neutral fraction. All polysaccharide fractions were mitogenic to cultured spleen cells of C57BL/6 mice. However, FA was different from FN in the co-mitogenicities with lectin mitogens. Both crude and acidic fractions potentiated remarkably the mitogenic activity of PHA-P or LPS in dose-dependent manner but neutral fraction enhanced only that of LPS. Three polysaccharide fractoins had no effect on that of Con A. These results suggest that the acidic fraction may stimulate B and T cells as well as macrophages while the neutral fraction may stimulate only B cells and macropages.

**Keywords**□*Panax ginseng*, immunomodulator, polysaccharide, antitumor activity, mitogenic activity, plaque forming cell, carbon clearance.

Among many physiological effectes of *Panax ginseng* C.A. Meyer which has long been utilized as an elixir of the life in oriental medicine, immunological effects have been known to be major effects. It has been also reported that ginseng extract and saponins enhanced immune responses[1-8]. However, no significant substances responsible for these effects have been isolated.

It has been reported that ginseng polysaccharide showed various biological effects including antitumor activity. The crude or purified polysaccharides exhibited antitumor activity and enhanced immune responses in tumor-bearing mice and cyclophosphamide-injected mice[9-12]. A number of hypoglycemic glycans (Panaxan A-U) was isolated from *Panax ginseng* roots in Korea, China and Japan[13-16]. Strongly acidic polysaccharide fractions showed the most potent anticomplementary activity among the water soluble and alkali-soluble fractions from the leaves and roots of *Panax ginseng*[17]. The macromolecular fraction containing mainly polysaccharides and saponin from fresh ginseng roots was reported to have mild mitogenic activity in human cord blood lymphocytes[18]. It was found that an acidic polysaccharide inhibited significantly toxohormon-L-induced lypolysis[19] and Sanchinan A from roots of *Panax notoginseng* activated reticuloendotherial system[20].

As mentioned above, ginseng polysaccharides are recognized to be important substances responsible for immunostimulating effect of *Panax ginseng*.

In this study, we have compared antitumor and immunomodulating activities of the neutral polysaccharide fraction with those of the acidic polysaccharide fraction from *Panax ginseng*.

## MATERIALS AND METHODS

### *Animals*

Male ICR mice, weighing 22-26g, 5-6 weeks old and male C57BL/6 mice, 12-14 weeks old were sup-

*To whom all correspondence should be addressed.

plied by our Animal Breeding Unit. They were housed in a room at $22 \pm 2°C$ with $55 \pm 5\%$ relative humidity and given a defined laboratory rodent chaw (Sam Yang Ind. Ltd.) and municipal tap water *ad libitum*.

### *Extraction and fractionation of water-soluble polysaccharides*

The powdered white ginseng (9 kg) prepared from the roots of *Panax ginseng* C.A. Mayer which was cultivated in Jungpyong Experiment Station of the Korea Ginseng and Tabacco Research Institute, Chungbuk Province, was defatted six times with aquous 85% ethanol (40 *l*). The remaining residues were extracted three times with hot water (80 *l*). The extracts were combined, concentrated to suitable volume and then dialyzed against running water for 3 days and distilled water for 1 day. After the non-dialyzate portion was centrifuged to remove insoluble materials, the resulting supernatant was precipitated with 5-6 volumes of ethanol. The precipitate, crude polysaccharide fraction (FO, 120g), was dried with acetone and then ether. FO, brown powder (3g) was chromatographed through a column ( $\phi$ 3 × 20 cm) of DEAE cellulose anion exchange ($Cl^-$ form) with water elution to be seperated into non adsorbed fraction, neutral polysaccharide fraction (FN, 1.2g) as a white powder after lyophilization and adsorbed fraction, which was eluted with 2 M NaCl solution. The NaCl elute was dialyzed aganist running water and lyophilized to give acidic polysaccharide fraction (FA, 1.0g) as a brownish powder.

### *General methods*

Total carbohydrate, uronic acid and protein contents were determined by anthrone test[21], carbazole assay[22] and Lowry-Folin method[23], using glucose, glucuronic acid and bovine serum albumin as respective standards.

### *Antitumor activity*

Antitumor activity was evaluated against the solid form of sarcoma 180 tumor. Tumor cells ($1 \times 10^6$/mouse) were inoculated subcutaneously into the left groin of ICR mice. After one day of tumor inoculation, each fractions (FO, FA and FN) were intraperitoneally administered once daily for 10 consecutive days. On day 25 the mice were sacrificed and tumor weights were measured. Tumor inhibition ratio (%) was calculated as follows:

$$\left(1-\frac{\text{average tumor weight of treated group}}{\text{average tumor weight of the control group}}\right)\times 100$$

### *Mitogenic activity*

The spleens were aseptically removed from C57BL/6 mice sacrificed by cervical dislocation, gently teased in ice-cold Hank's Buffered Salt Solution (HBSS, pH 7.2). The cell suspension was passed through a nylon mesh and centrifuged at 400g for 5 min. The cell pellet was suspended in 10 m*l* of Tris-buffered 0.83% $NH_4Cl$ (pH 7.2) to lyse red cells, incubated at 37°C for 5 min and washed twice with RPMI 1640 medium containing 25 mM Hepes, 10% fetal calf serum, $10^{-5}$ M 2-mercaptoethanol, streptomycin (100 mg/*l*) and penicillin (100,000 units/*l*) by centrifugation at 400g for 5 min. Viability was assessed with the trypan blue dye exclusion test. All assays were performed in triplicate culture. Each sample (50 $\mu$ *l*) dissolved in HBSS and 1 m*l* of spleen cell suspension was placed in tissue culture tube (Falcon, #2054). The cultures were incubated at 37°C in a $CO_2$ incubator for 72 hours. At 24 hours before cell harvesting, 0.5 $\mu$Ci of (6-$^3$H)-thymidine (2 Ci/mmol, Amersham) was added to the culture in a volume of 50 $\mu$ *l*. At the end of the incubation, the cultured spleen cells were harvested on the glass fiber filter, washed twice with 2 m*l* of ice-cold HBSS, lysed with 10 m*l* of ice-cold 10% TCA, and washed with 10 m*l* of 95% ethanol. The radioactivity was measured on liquid scintillation counter (Beckman, LS1800). Stimulation index was calculated by the following equation:

$$\text{S.I.} = \frac{\text{mean cpm in each experimental group}}{\text{mean cpm in control group}}$$

To determine co-mitogenic effect, spleen cells were cultured with each fraction in the presense of concanavalin A (ConA, 1 $\mu$g/m*l*, Sigma Co.), phytohemaglutinin-P (PHA-P, 5 $\mu$g/m*l*, Sigma Co.) or lipopolysaccharide (LPS, 2 $\mu$g/m*l*, Sigma Co.) as discribed above.

### *Hemolytic plaque forming cells (PFC) assay*

The PFC responses to sheep red blood cells (SRBC) was determined by the method of Cunningham and Szenberg[24] with a slight modification. Each sample was administered intraperitoneally to ICR mice for 6 days, starting from day 1. On day 2, mice were sensitized with $1 \times 10^7$ SRBC via tail vein and challenged with $1 \times 10^8$ SRBC intradermally on day 7. Mice were killed by cervical dislocation on day 8 (i.e., one day after challenge) and the weights of liver, spleen and thymus were measured. The single spleen cell suspension was prepared in ice-cold HBSS as discribed above. Two microchambers with the volume capacity of 45 $\mu$ *l*, covered with cover glass, were made on slide glass. The suspension of indicator red cells in HBSS was prepared by mixing 500 $\mu$ *l* of 50% (v/v) SRBC

and 1000 $\mu l$ of guinea pig complement (Sigma Co.) and kept in an ice bath for 30 min. A reaction mixture was composed of 435 $\mu l$ of spleen cell suspension and 100 $\mu l$ of suspension of indicator red cells, packed into microchamber and then microchambers were sealed with the mixture of paraffin and wax (1:1). After incubation at 37°C for 1 hr, the number of hemolytic plaques was counted under a low power microscope. PFC/$10^6$ spleen cells and PFC/spleen were calculated by means of the following equations:

$$\text{PFC}/10^6 \text{ spleen cells} = \frac{N}{C \cdot V_m \cdot a} \, 10^6$$

$$\text{PFC/spleen} = \frac{\text{PFC}}{10^6 \text{ spleen cells}} \cdot C \cdot V_s$$

a: the ratio of spleen cell suspension volume in reaction mixture (435/535)
N: the number of plaques counted in a microchamber
C: the count of spleen cells in 1 m$l$ of spleen cell suspension
$V_m$: the volume of incubation mixture filled into a microchamber
$V_s$: total volume of spleen cell suspension (m$l$)

### *Phagocytic activity*

Phagocytic function was determined by the method of Biozzi *et al*[25].
One day after 6 consecutive sample administrations once daily, a suspension of carbon particle (Rotring Drowing Ink, Germany) in 1% gelatin solution (1:5) was injected to ICR mice via tail vain (0.1 m$l$/10g body weight). A blood sample 20 $\mu l$ was taken from the retro-orbital plexus with heparinized capillary at 2 and 10 min after carbon injection and transferred into 2 m$l$ of 0.1% $Na_2CO_3$. The absorbance at 600 nm was measured. The phagocytic index (K) and corrected phagocytic index ( $\alpha$ ) were calculated by means of the following equation:

$$K = \frac{\log OD t_1 - \log OD t_2}{t_2 - t_1} \qquad \alpha = \sqrt[3]{k} \, \frac{P_c}{P_o}$$

$OD_1$, $OD_2$: the optical density at time $t_1$ and time $t_2$, respectively
$P_c$: body weight
$P_o$: liver weight + spleen weight

### *Statistical analysis*

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

## RESULTS

The chemical composition of neutral (FN) and acidic polysaccharide fraction (FA) prepared from crude ginseng polysaccharides by fractionation on DEAE-cellulose anion exchange ($Cl^-$form) were shown in Table I. FN and FA were composed of 85% carbohydrate, 15% protein and a trace amount of uronic acid, and 28.4% carbohydrate, 10% protein and 29.0% uronic acid, respectively. Thus, it was identified that FN was a neutral polysaccharide fraction and FA was an acidic polysaccharide fraction containing uronic acid.

As shown in Table II, the i.p. administration of FO (300 mg/kg), FN (100 mg/kg) or FA (100 mg/kg) into ICR mice bearing a solid form of sarcoma 180 suppressed tumor growth by 42%, 36%, and 47%, respectively. The more purified fraction, FN and FA, showed higher antitumor activity at lower dose as compared to that of FO, crude polysaccharide fraction.

To examine effects of each polysaccharide fractions on the weight of immune-related organs in ICR mice, liver, spleen and thymus were weighed. As shown in Table III, all of polysaccharide fractions had no effect on body weight, but the ratio of liver to body weight was significantly increased by 1.19 and 1.18 fold by FN and FA, respectively, and that of thymus to body weight showed the tendency to increase but it was not statistically significant. Also, the spleens were markedly enlarged by i.p. injections of each polysaccharide fraction as compared with the control even though the increase of organ weights might be in part due to immunizations. At a dose of 100 mg/kg, the increase in the weight of spleen by FA was greater than that by FN.

To assess the polyclonal B cell activity by each polysaccharide fraction, PFC response to SRBC was determined *in vivo*. The number of direct PFC, which represents the number of immunoglobulin M(IgM)-

**Table I. Chemical composition of polysaccharide fractions from *Panax ginseng***

| Fraction | Carbohydrate[a] | Protein[b] | Uronic acid[c] |
|---|---|---|---|
| FN | 85.0% | 15.0% | trace |
| FA | 28.4% | 10.0% | 29.0% |

[a] Anthrone-$H_2SO_4$ method uses glucose as standard.
[b] Lowry-Folin method uses bovine serum albumin as standard.
[c] Carbazole assay uses glucuronic acid as standard.

**Table II. Antitumor activities of polysaccharide fractions from *Panax ginseng*[a]**

| Group | Dose (mg/kg) | No. of mice | Body weight[b] change (g) | Tumor weight (g, mean ± S.D.) | Inhibition[b] ratio (%) | Significance[c] (p<) |
|---|---|---|---|---|---|---|
| Control | – | 12 | +6.7 | 2.03 ± 1.24 | – | – |
| FO | 100 | 12 | +5.2 | 1.62 ± 1.16 | 20 | n.s.[d] |
| | 300 | 13 | +5.1 | 1.17 ± 0.80 | 42 | 0.025 |
| Control | – | 15 | +7.5 | 1.51 ± 0.93 | – | – |
| FN | 20 | 12 | +8.1 | 1.54 ± 0.79 | −2 | n.s. |
| | 100 | 12 | +9.0 | 0.96 ± 0.42 | 36 | 0.05 |
| FA | 20 | 12 | +8.5 | 1.24 ± 0.72 | 18 | n.s. |
| | 100 | 13 | +7.8 | 0.80 ± 0.72 | 47 | 0.025 |

[a]Sarcoma 180 cells ($1 \times 10^6$) were inoculated subcutaneously to the left groin of ICR mice. Each sample was intraperitoneally adminstered 24 hr after inoculation as saline solution for 10 consecutive days.
[b]Body weight change and inhibition ratio were determined at day 10 and 25 after tumor inoculation, respectively.
[c]The significance was evaluated according to Student's t-test against each control group.
[d]Not significant.

**Table III. Effects of ginseng polysaccharide fractions on the weight of immunoorgans in ICR mice**

| Group | Dose (mg/kg) | Body weight (g) | % Body weight Liver | Spleen | Thymus |
|---|---|---|---|---|---|
| Control | – | 29.2 ± 2.0 | 6.74 ± 0.42 (1.00) | 0.64 ± 0.07 (1.00) | 0.24 ± 0.07 (1.00) |
| FO | 100 | 28.0 ± 1.8 | 7.35 ± 0.42 (1.09)* | 0.89 ± 0.12 (1.39)*** | 0.26 ± 0.05 (1.08) |
| FO | 300 | 29.8 ± 1.6 | 7.72 ± 0.80 (1.15)* | 1.29 ± 0.18 (2.02)*** | 0.27 ± 0.06 (1.13) |
| Control | – | 28.5 ± 1.1 | 7.09 ± 0.22 (1.00) | 0.59 ± 0.09 (1.00) | 0.25 ± 0.05 (1.00) |
| FN | 100 | 29.5 ± 0.9 | 8.41 ± 0.18 (1.19)*** | 0.81 ± 0.04 (1.37)*** | 0.27 ± 0.03 (1.08) |
| FA | 100 | 29.6 ± 0.7 | 8.39 ± 0.92 (1.18)* | 0.95 ± 0.05 (1.61)*** | 0.28 ± 0.05 (1.12) |

Each fraction was administered intraperitoneally for 6 consecutive days as a saline solution. The detailed experimental schedule was indicated in Table IV.
After 2 day of last adminstration weights of immunoorgans were measured.
Each value is mean ± S.D. of 5 to 6 animals.
The value in parenthesis is expressed relative to the control value (1.00) Significant difference from each control; * $p<0.05$, ** $p<0.01$, *** $p<0.005$.

producing lymphocytes, was demonstrated in Table IV. At a dose of 100 mg/kg of FO, FN and FA, PFC/$10^6$ spleen cells showed increases by 2.18, 2.49 and 3.49 fold in comparison with each control, respectively. Also, the i. p. injection of the polysaccharide fraction was found to increase total number of spleen cells as in the weights of spleens. FA exhibited higher effects on PFC to SRBC and total number of spleen cells than FN. These results indicated that ginseng polysaccharide fractions, FO and FN as well as FA differentiated B-cells to antibody-secreating cells polyclonally *in vivo*.

To determine whether ginseng polysaccharide fractions have mitogenic activity, different concentrations (5-250 $\mu$g/ml) of each fraction were added to the cultured spleen cells of C57BL/6 mice and the incoporation of ($^3$H)-thymidine into these cells *in vitro* was measured. As shown in Fig. 2, all fractions showed significant mitogenic activity against spleen cells of C57BL/6 mice in a dose-dependent manner by stimulating thymidine incoporation. Although FO and FA were effective at the dose of 5 $\mu$g/ml, FN was effective at a dose of 100 $\mu$g/ml. However, the mitogenic activities were very low in comparison with that of LPS, PHA-P or Con A using as a positive control.

In the presence of bacterial LPS (2 $\mu$g/ml) which

**Table IV. Polyclonal B-cell activity of polysaccharide fractions from *Panax ginseng* in ICR mice**

| Group | Dose (mg/kg) | Total spleen cells ($\times 10^8$) | PFC/$10^6$ spleen cells | PFC/spleen ($\times 10^2$) |
|---|---|---|---|---|
| Control | – | 1.17 ± 0.33 (1.00) | 22.6 ± 7.5 (1.00) | 27.1 ± 14.5 (1.00) |
| FO | 100 | 1.83 ± 0.36 (1.56)** | 49.3 ± 19.2 (2.18)** | 80.0 ± 33.0 (2.95)** |
| FO | 300 | 2.96 ± 0.71 (2.53)*** | 61.3 ± 10.0 (2.71)*** | 149.0 ± 38.0 (5.49)*** |
| Control | – | 0.99 ± 0.44 (1.00) | 27.7 ± 8.1 (1.00) | 27.2 ± 11.9 (1.00) |
| FN | 100 | 1.50 ± 0.11 (1.52)* | 69.1 ± 14.7 (2.49)*** | 109.3 ± 30.0 (4.02)*** |
| FA | 100 | 2.04 ± 0.38 (2.06)*** | 96.7 ± 28.2 (3.49)*** | 176.7 ± 36.7 (6.50)*** |

Each fraction was administered intraperitoneally on day 1-6 as a saline solution.
On day 2, mice were sensitized with $1 \times 10^7$ SRBC into the tail vein, on day 7 intradermally challenged with $1 \times 10^8$SRBC and on day 8 PFC were determined by Cunningham's method with a slight modification.
Each value represents mean ± S.D. of 5 mice.
The value in parenthesis is expressed relative to the control value (1.00).
Significant difference from each control; * $p<0.05$, ** $p<0.01$, *** $p<0.005$.

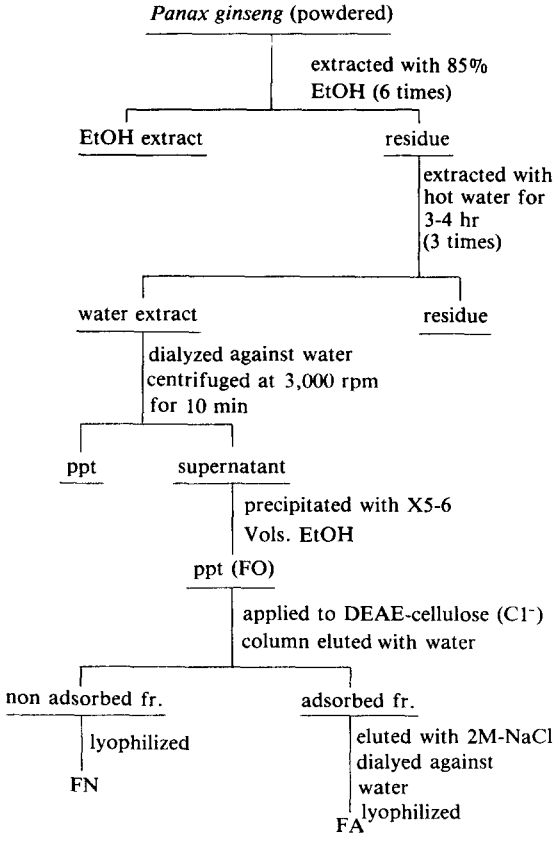


**Fig. 1. Fractionation of water soluble polysaccharides from *Panax ginseng*.**

is a B cell mitogen, Con A (1 μg/ml) or PHA-P (5 μg/ml) which are T cell mitogens, the effects of

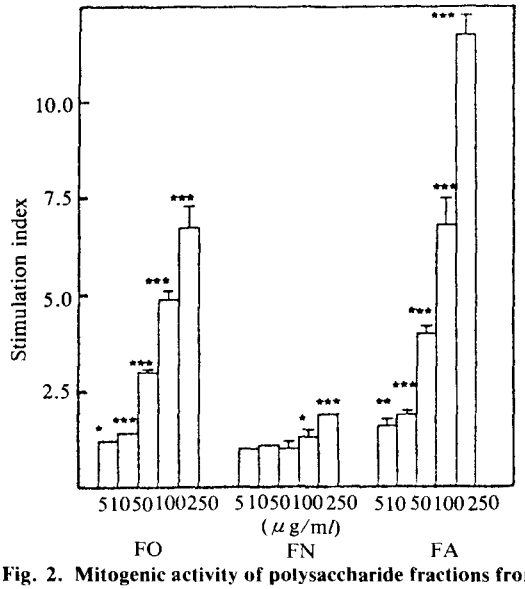


**Fig. 2. Mitogenic activity of polysaccharide fractions from *Panax ginseng* on cultured spleen cells of C57BL/6 mice.**

Spleen cells ($1 \times 10^6$/culture) were cultured for 72 hr at 37°C in a $CO_2$ incubator. 24 hr before harvesting, 0.5 μci of ($^3$H)-thymidine was added to the culture medium in a volume of 50 μl.

$$\text{S.I.} = \frac{\text{mean cpm in each experimental group}}{\text{mean cpm in control group}}$$

The mean cpm ± S.D. of the control group was 5688 ± 802 (1.00). As positive controls, mitogens showed the following S.I.: ConA, 36.63 at 5 μg/ml; LPS, 11.95 at 2 μg/ml; PHA-P, 5.98 at 5 μg/ml. Significant difference from the control group; * $p<0.05$, ** $p<0.01$, *** $p<0.005$.

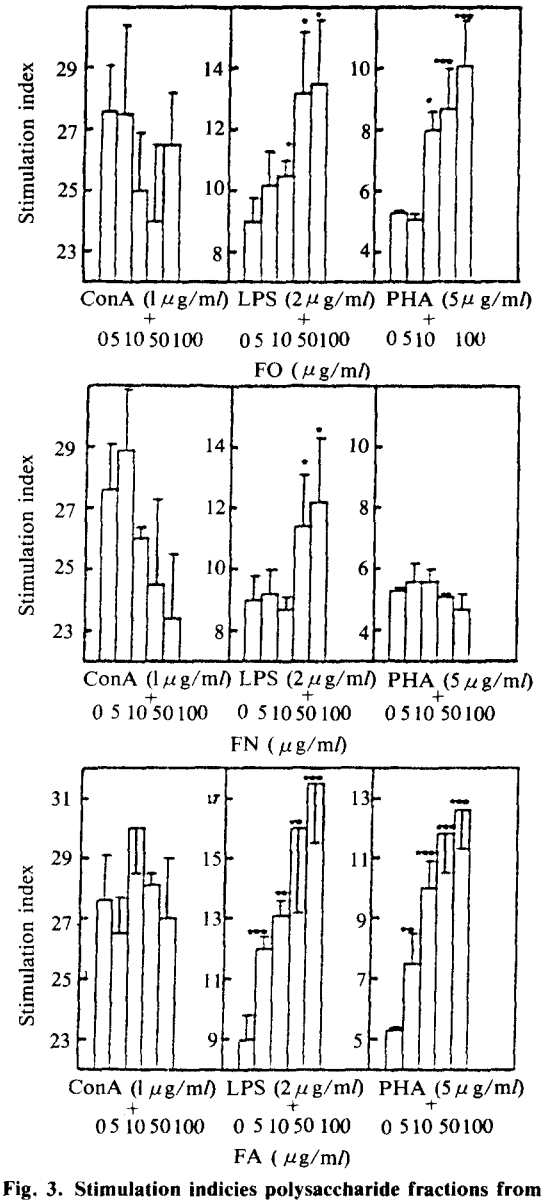


**Fig. 3. Stimulation indicies polysaccharide fractions from *Panax ginseng* in the presence of various mitogens.** Spleen cells ($1 \times 10^6$/culture) from C57BL/6 time were cultured for 72 hr at 37°C in a $CO_2$ incubator. 24 hr before harvesting, 0.5 μCi of ($^3$H)-thymidine was added to the culture medium in a volume of 50 μl.

$$\text{S.I.} = \frac{\text{mean cpm in each experimental group}}{\text{mean cpm in control group}}$$

The mean cpm ± S.D. of the control group was 4717 ± 590

Significant difference from the control group; * $p<0.05$, ** $p<0.01$, *** $p<0.005$.

each fraction on mitogenecity of each mitogen were examined. As shown in Fig. 3, both FO and FA enhanced dose-dependently the mitogenecity of LPS and PHA-P, however FN enchanced only LPS stimulation at over 50 μg/ml. All fractions had no effects on mitogenic activity of Con A. Also FA showed the stronest effect on the mitogenic and co-mitogenic activity. From these results, it was observed that FO, FN and FA were active as a mitogen by itself and potentiated the activity of lectin mitogens in T cells and/or B cells.

The effect of polysaccharide fractions on reticuloendotherial system (RES) was assessed by comparing the clearance time of carbon particles from blood stream. As shown in Table V, phagocytic index (K) was remarkably increased by all fraction, suggesting that ginseng polysaccharide fractions activated nonspecific phagocytosis of splenic sinus macrophages and hepatic Kupffer cells.

## DISCUSSION

The antitumor polysaccharides from several fungi, such as lentinan from *Lentinus edoeds*[26], schizophyllan from *Schizophyllum commune*[27], and Krestin (PS-K) from *Coriolus versicolor*[28], are now used clinically for cancer therpy and it is well known that their antitumor activity is due to the activation of immune system.

In recent years, polysaccharides have emerged as an important class of bioactive natural products. Also ginseng polysaccharides have been shown to have immunostimulating activities and various biological effects.

In this study, it was observed that FO (crude fraction), FN (uronic acid free fraction) and FA (acidic fraction cantaining 29% uronic acid) had antitumor activity against the solid form of sarcoma 180 and marked immunostimulating activities, such as polyclonal B cell activity, mitogenic activity and activation of RES. FA was different from FN in the comitogenic activity with lectin mitogens.

These findings suggest that immunomodulating effects of FA may result from the stimulation of B and T cells as well as macrophages but FN may stimulate only B cells and macropages.

In the view of this point, it might be expected that FA is more effective in immunomodulating activities than FN. Also, higher effective immunomodulating activities of FA were in agreement with the report[29] that polysaccharides containing uronic acid have been found to be better immunostimulants than uronic acid free polysaccharides.

In the present study, it was observed that ginseng

**Table V. Effects of polysaccharide fractions from *Panax ginseng* on carbon clearance activity in ICR mice**

| Group | Dose (mg/kg) | Phagocytic index ($\times 10^{-2}$) | Corrected phagocytic indexd |
|---|---|---|---|
| Control | – | 1.31 ± 0.42 (1.00) | 3.73 ± 0.35 (1.00) |
| FO | 100 | 4.33 ± 0.74 (3.31)*** | 4.36 ± 0.42 (1.17)* |
| FO | 300 | 6.79 ± 0.56 (5.18)*** | 4.96 ± 0.32 (1.33)*** |
| FN | 100 | 3.15 ± 0.89 (2.40)*** | 4.13 ± 0.15 (1.11)* |
| FA | 100 | 6.02 ± 0.65 (4.60)*** | 5.04 ± 0.54 (1.35)*** |

Each fraction was administered intraperitoneally for 6 consecutive days.
After 24 hr of last administration carbon cleance was determined by the method of Biozzi *et al.*
Each value represents mean ± S.D. of 4 mice.
Significant difference from the control group; * $p<0.05$, *** $p<0.005$.

polysaccharide fractions had more potent immunomodulating activity than antitumor activity. These results were consistent with the notion[29] that polysaccharides isolated from fungi usually show antitumor activity, while higher plant polysaccharides possess immunostimulatory activity.

It was reported that the activity of antitumor glucans from fungi belonging to Basidiomycetes and various species of *Grifora* was due to a 6-brnached (1→3) β-glucan[30-34]. For more detailed studies on the relationship among the structure, antitumor and immunomodulating activity of ginseng polysaccharides, further purifications of not only water-soluble but also alkali-soluble polysaccharide fractions are required.

In conclusion, antitumor activity of ginseng polysaccharide fractions is host mediated by stimulating immune system and the acidic polysaccharide fraction has more potent immunomodulating activity than the neutral fraction.

## LITERATURE CITED


1. Ha, T.Y., Lee, J.H. and Kim, S.H.: Effect of *Panax ginseng* on the graft-versus-host reaction, production of leucocyte migration inhibitory factor and expulsion of aldult *Trichinealla Spiralis* in mice. *J. Korean Soc. Microbiol.* **21,** 133 (1986).
2. Jie, Y.H., Cammisuli, S. and Baggiolini M.: Immunomodulatory effects of *Panax ginseng* C.A. Meyer in the mouse. *Agents and Actions* **15,** 386 (1984).
3. Singh, V.K., Agarwal, S.S. and Gupta, B.M.: Immunomodulatory activity of *Panax ginseng* extract. *Panta Medica* **50,** 462 (1984).
4. Matsuda, H., Hasegawa, T. and Kubo, M.: Pharmacological study on *Panax ginseng* C.A. Meyer, VII. Protective effect of red ginseng on infection (1) On phagocytic activity of mouse reticuloendotherial system. *Yakugaku Zasshi* **105,** 948 (1985).
5. Kim, M.J. and Jung, N.P.: The effect of ginseng saponin on the mouse immune system, *Korean J. Ginseng Sci.* **11,** 130 (1987).
6. Matsuda, H., Kubo, M., Tani, T., Kitaguwa, I. and Mizuno, M.: Pharmacological study on *Panax ginseng* C.A. Meyer (IX). Protective effect of red ginseng on infection (2) on phagocytic activity of mouse reticuloendotherial system. *Shoyakugaku Zasshi* **41,** 131 (1987).
7. Park, H.W., Kim, S.C. and Jung, N.P.: The effect of ginseng saponin fractions on humoral immunity of mouse. *Korean J. Ginseng Sic.* **12,** 63 (1988).
8. Kim, M.N. and Jung, N.P.: The effect of ginseng saponin fractions on NK activity in mice. *Korean J. Ginseng Sci.* **13,** 223 (1989)
9. Moon, C.K., Sim, K.S., Lee, S.H., Park, K.S., Yun, Y.P., Ha, B.J. and Lee, C.C.: Antitumor activity of some phytobased polysaccharides and their effects on the immune function. *Arch. Pharm. Res.* **6,** 123 (1983) .
10. Wang, B.X., Lhi, J.C. and Cui, A.J.: Study on anti-tumor activity of ginseng polysaccharides, 5th, Int. Ginseng Symp. (Abstract) (1988).
11. Quian, B.C., Zang, X.X., Li, B., Xu, C.Y. and Peng, X.Y.: Effects of ginseng polysaccharides on tumor and immunological function in tumor-bearing mice. *Acta. Pharmacol. Sin.* **8,** 277 (1987).
12. Yuan, X.N., Shang, X.H., Jin, M.L. and Shang, K.: Effects of ginseng root polysaccharides on cyclophoslphamide-suppressed immunity. *Shenyang Yaoxueyuan Xeuebao* **3,** 162 (1986).
13. Konno, C., Sugiyama, K., Kano, M., Takahashi, M. and Hikino, H.: Isolation and hypoglycemic activity of panaxans A, B, C, D and E, glycans of *Panax ginseng* roots. *Planta Medica* **50,** 443

(1984).
14. Hikino, H., Oshima, Y., Suzuki, Y. and Konno, C.: Isolation and hyploglycemic activity of panaxans F. G. H. I. J. K. and L, glycans of *Panax ginseng* roots. *Shoyakigaku Zasshi* **39**, 331 (1985).
15. Konno, C. and Hikino, H.: Isolation and hypoglycemic activity of panaxans M, N, O, and P, glycans of *Panax ginseng* roots. *Int. J. Crude Drug. Res.* **25**, 53 (1987).
16. Kanno, C., Murakami, M., Oshima, Y. and Hikino, H.: Isolation and hypoglycemic activity of panaxans Q, R, S, T and U, glycans of *Panax ginseng* roots. *J. Ethnopharmacol.* **14**, 69 (1985).
17. Gao, Q.P., Kiyohara, H., Cyong, J.C. and Yamada, H.: Chemical properties and anti-complementary activities of polysaccharide fractions from roots and leaves of *Panax ginseng*. *Planta Medica* **55**, 9 (1989).
18. Eun, S.M., Hung, N.K., Nam, L.K. and Cheung, K.Y.: Growth promoting activity of a macromolecular fraction from fresh ginseng. *Korean J. Ginseng Sci.* **13**, 215 (1989).
19. Lee, S.D. and Okuda, H.: Effect of acidic polysaccharide of Korean red ginseng on lipolytic action of toxohormone-L from cancerous ascites fluid. *Korean J. Ginseng Sci.* **14**, 67 (1990).
20. Ohtani, K., Mizutani, K., Hatono, S., Kasai, R., Sumino, R., Shiota, T., Ushi, M., Fuwa, T. and Tanaka, O.: Sanchinan-A, a reticuloendothelial system activating arabinogalactan from Sanchi-Ginseng (Roots of *Panax notoginseng). Planta Medica* **53**, 166 (1987).
21. Dimler, R.J., Schaefer, W.C., Wise, C.S. and Rist, C.E.: Quantitative paper chromatography of D-glucose and its oligosaccharides. *Anal. Chem.* **24**, 1411 (1952).
22. Chaplin, M.F. andd Kennedy, J.F.: *Carbohydrate Analysis,* 1st ed., IRL Press, Oxford, p. 5 (1987).
23. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurment with the folin phenol reagent. *J. Biol. Chem.* **193**, 265 (1951).
24. Cunningham, A.J. and Szenberg, A.: Futher improvements in the plaque technique for detecting single antibody forming cells. *Immunology* **14**, 599 (1968).
25. Biozzi, G., Benacerraf, B., Stiffel, C. and Halpern, B.N.: Quantitative study of the granulopetic activity of the reticuloendotherial system. *Br. J. Exp. Patho.* **34**, 447 (1954).
26. Chihara, G., Hamuro, J., Maeda, Y., Ayai, Y. and Fukuoka, F.: Fractionation and purification of the polysaccharides with marked anti-tumor activity, especially lentinan from *Lentinus edodes* (Berk.) *Sing. Cancer Res.* **30**, 2776 (1970).
27. Komatsu, N., Okubo, S., Kikumoto, S., Kimura, K., Saito, G. and Sakai, S.: Host mediated antitumor action of schizophyllan, a glucan produced by *Schizophyllum Commune. Gann* **60**, 137 (1969).
28. Tsugagoshi, S. and Ohashi, F.: Protein-bound polysaccharide preparation, PS-K, effective against mouse sarcoma 180 and rat ascites hepatoma AH-13 by oral use. *Gann* **65**, 557 (1974).
29. Srivastava, R. and Kulshreshtha, D.K.: Bioactive polysaccharides from plants. *Phytochem.* **28**, 2877 (1989).
30. Ohno, N., Sutzuki, I., Oikawa, S., Sato, K., Miyazaki, T. andd Yadomae, T.: Antitumor activity and structural charicterization of glucans extracted from cultured fruit bodies of *Grifola frondasa. Chem. Pharm. Bull.* **32**, 1142 (1984).
31. Iino, K., Ohno, N., Suzuki, I., Sato, K., Oikawa, S. and Yadomae, T.: Structure-function relationship of antitumor $\beta$-1, 3-glucan obtained from matted mycelium of cultured *Grifola frondosa. Chem. Pharm. Bull.* **33**, 4950 (1985).
32. Adachi, Y., Ohno, N., Ohsawa, M., Sata, K., Oikawa, S. and Yadomae, T.: Physicochemical properties and antitumor activities of chemically modified derivatives of antitumor glucan "Grifloran LE" from *Grifola frondosa. Chem. Pharm. Bull.* **37**, 1838 (1989).
33. Kang, C.Y., Shim, M.J., Choi, E.C., Lee, Y.N. and Kim, B.K.: Studies on antitumor components of Korean Basidiomycetes. Mycelial culture and antitumor components of *Ganoderma lucidum. Kor. Biochem. J.* **14**, 101 (1981).
34. Kim, B.K., Chung, H.S., Chung, K.S. and Yang, M.S.: Studies on the antineoplastic components of Korean Basidiomycetes. *Kor. J. Mycol.* **8**, 107 (1980).