Immunomodulating Activity of Ginsenoside Rg1 from *Panax Ginseng*

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Abstract—The immunomodulatory activity of ginsenoside Rg1 from *Panax ginseng* was studied in mice using sheep red cells as the antigen. It was found that ginsenoside Rg1 at a dose of 10 mg/kg administered for three consecutive days before immunization increased the number of spleen plaque-forming cells, the titers of sera hemagglutinins as well as the number of antigen-reactive T-cells. Ginsenoside Rg1 also increased the number of T-helper cells with respect to the whole T-cell number and the splenocyte natural killer activity. Ginsenoside Rg1 induced an augmentation of the production of IL-1 by macrophages and exerted a direct mitogenic effect on microcultured thymus cells. Ginsenoside Rg1 also partly restored the impaired immune reactivity by cyclophosphamide treatment.

Extracts of *Panax ginseng* have been a valuable and important folk drug in many Asian countries such as China, Korea and Japan for the past 2000 years. Among the variety of pharmacological effects of ginseng is its ability to replenish vital energy and strengthen body resistance (1). It has been shown that ginseng prevents stress reactions, facilitates learning and improves memory, especially in cases of memory deficits occurring naturally in old laboratory animals (2–5). The beneficial effects of ginseng in the elderly suggest that ginseng has an influence on not only the brain but also on other systems involved in the homeostasis of the organism, e.g. the immune system. Sing et al. were the first to show experimentally that *Panax ginseng* is capable of enhancing the immune response in mice (6, 7).

The main active ginseng constituents are triterpenoid saponins, ginsenosides, which have been isolated and chemically characterized by Shibata’s group (8, 9). It has been found that they possess a broad spectrum of different but in some cases opposite pharmacological activities. In spite of the presumed immunostimulating activity of ginseng (10), few studies have been done to elucidate the effect of whole ginseng extracts and those of individual ginsenosides on the immune system.

The aim of the present investigation was to

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Fig. 1. Chemical structure of ginsenoside Rg1 from *Panax ginseng*.
evaluate the effect of one of the main active ginseng components, ginsenoside Rg1 (Fig. 1), on the immune response.

Materials and Methods

1. Animals
We employed BALB/c mice for the in vitro study and ICR mice for the in vivo experiments; the animals weighed 16–18 g and were given free access to food (standardized diet of pellets) and water.

2. Drug treatment
2.1. The purified ginsenoside Rg1 was a generous gift from Takeda Chemical Industries, Osaka. In preliminary studies, we tried two different routes of ginsenoside Rg1 administration, i.p. and i.v., and the resultant effects on the immune reactivity were identical. In all the in vivo studies, the drug was administered i.p. at a dose of 10 mg/kg for three consecutive days before immunization (the last injection was made on the day of immunization); dosage and method of administration suggested by the doses used in the experiments of Singh et al. with extracts of Panax ginseng (7) and in the study of Saito (1) about the effect of i.p. administration of ginsenoside Rg1 on learning and memory. A control group of mice was included in all the experiments and the animals received phosphate buffer saline (PBS) only.

2.2. Pretreatment of mice with cyclophosphamide: Cyclophosphamide (Serva) (200 mg/kg) was injected i.p. into mice on the first day of the ginsenoside Rg1 treatment.

3. Immunological tests
3.1. Sheep red blood cells (SRBC): SRBC were used as antigen. Both treated and control mice were injected i.p. with 5×10⁸ SRBC.

3.2. Preparation of spleen cell suspension: Spleens were aseptically taken from mice and gently crushed and separated into single cells by squeezing in RPMI-1640 medium (Gibco). The cells were obtained by centrifugation at 400×g for 10 min. The collected cells were washed twice with RPMI-1640 medium, followed by resuspension in an appropriate volume.

3.3. Hemagglutinating antibody assay: Groups of 5 mice receiving ginsenoside Rg1 for three consecutive days before SRBC and groups of control mice were killed 3, 5, 7, 10 and 15 days after immunization. Sera samples of the treated and control animals were pooled and inactivated at 56°C for 30 min. Two-fold dilutions of sera samples were made in PBS in microtiter plates and mixed (1:1) with a 1.0% suspension of SRBC in PBS. End points of agglutination were noted after overnight incubation at 4°C.

3.4. Plaque-forming cell (PFC) response: Direct antibody forming cells in the spleens of test and control mice were estimated using the hemolytic technique of Cunningham and Szenberg (11). Briefly, 0.2 ml of a 6.0% suspension of SRBC, 0.2 ml of spleen cell suspension (5×10⁶) and 0.2 ml of guinea pig complement (Institute of Infectious and Parasitic Diseases, Sofia) diluted 1:7 in PBS were mixed. The resultant mixture (0.2 ml) was introduced into glass plate cameras (75/25 mm). The hermetically closed cameras were incubated for 30 min at 37°C and the plaques formed were counted.

3.5. Antigen-reactive T-cells assay: Antigen-reactive T-cells were estimated by the immunocytoadherent technique of Mendes et al. (12). A 0.2-ml aliquot of spleen cell suspension (5×10⁶) was mixed with 0.2 ml of a 1.0% suspension of SRBC, both diluted in Parker medium (Difco), containing 0.1% EDTA (Serva) and 0.8% human serum albumin (Serva). After mixing, the suspensions were centrifuged at 200×g for 5 min at 4°C and were incubated overnight at 4°C. The suspension were carefully resuspended with a Pasteur pipette; one drop was added to a microscope slide and counted microscopically to determine the number of lymphocyte-forming rosettes. A rosette was usually defined as a lymphocyte to which at least three SRBC adhere.

3.6. Cell-mediated immune response: The cell-mediated immune reaction was evaluated by the popliteal lymph node indexes (13). For this purpose, 0.1 ml of 5.0% SRBC suspension were injected subcutaneously into one of the hind foot pads of both control and treated mice. After 7 days, 0.1 ml of a 1.0% suspension of SRBC were injected into the contralateral hind foot pad. After 24 hr, the mice were killed and then the popliteal lymph nodes were removed and weighed. The ratio of the weight of the treated to the untreated
3.7. T-helper cell response: T-helper cells were determined by the number of theophylline-independent antigen-binding cells according to Shore et al. (14). Briefly, 0.2 ml of spleen cell suspension (5 x 10^6) was incubated for 60 min at 37°C with RPMI-1640 medium containing 0.02 M theophylline (Fluka). The cell suspensions were then evaluated for rosette forming cells as described in section 3.5. The T-helper cell percentage was determined as the ratio of the number of the rosette forming cells in the presence of theophylline (theophylline-independent T-cells) to the number of the rosette forming cells in the absence of theophylline (antigen-reactive T-cells).

3.8. Natural killer (NK) cell activity: NK activity was measured as splenic cytolytic activity against chick erythrocytes as target cells (7). Briefly, chick erythrocytes at a concentration of 1 x 10^6 were incubated for 18 hr in RPMI-1640 medium containing 148 KBq/ml [6-³H]-uridine (specific activity 558.7 GBq/mmol, Amersham). Splenocytes from both ginsenoside Rg₁ treated and control mice were taken 5 and 10 days after the last injection. These cells were placed in plastic dishes and incubated for 1 hr at 37°C, and the non-adherent cells were harvested and further used as effector cells. Both target and effector cells were treated with actinomycin D (Serva) (2 µg/ml) for 1 hr just before the lytic reaction. Splenocytes and target chick erythrocytes (cell ratio 100:1) were cultivated for 4 hr in RPMI-1640 medium containing ginsenoside Rg₁ (25 µg/ml and 50 µg/ml) or lipopolysaccharides (LPS) from E. coli 0111 (10 µg/ml). Incubation was continued for 24 hr; and thereafter, each culture supernatant was collected by centrifugation at 200 x g for 5 min, dialyzed against PBS, and then filtered and stored at -20°C until use.

3.9. Induction of interleukin-1 (IL-1): Peritoneal exudate cells (PECs) were collected from the peritoneal cavity of each of three mice, pooled and washed once with RPMI-1640 medium. PECs were resuspended in RPMI-1640 medium containing 5.0% fetal calf serum (FCS) (Gibco), 100 U/ml penicillin (Pharmahim, Bulgaria) and 100 µg/ml streptomycin (Pharmahim, Bulgaria). PECs (1 x 10^6) were placed into each well of round-bottomed tissue culture trays with 24 wells 2-hr incubation at 37°C in a humidified air containing 5.0% CO₂, the nonadherent cells were removed. Adherent cells were reincubated in the same RPMI-1640 medium and in RPMI-1640 medium containing ginsenoside Rg₁ (25 µg/ml and 50 µg/ml) or lipopolysaccharides (LPS) from E. coli 0111 (10 µg/ml). Incubation was continued for 24 hr; and thereafter, each culture supernatant was collected by centrifugation at 200 x g for 5 min, dialyzed against PBS, and then filtered and stored at -20°C until use.

4. Statistics

Results are shown as the mean±S.E. Statistical evaluation was performed using Student’s t-test.

Results

1. Effect of ginsenoside Rg₁ on PFC production in the spleen and serum antibody formation: The effects of the in vivo administration of ginsenoside Rg₁ on the num-
ber of PFCs and serum hemagglutinating titer on different days after SRBC immunization are shown in Tables 1 and 2. The PFC number was increased in the treated mice as compared to the control mice 5, 7 and 10 days after the immunization by 45.1, 34.5 and 113.1 percent, respectively (Table 1). The same tendency was seen in the titers of serum hemagglutinating antibodies (Table 2). In the treated group, the hemagglutinating titer was 2-fold higher than that of the control group on the 5th day, 8-fold higher on the 7th day and 2-fold higher on the 10th and 15th days after the immunization.

The same parameters of humoral immune response were estimated in mice pretreated with cyclophosphamide (Tables 1 and 2). The number of PFCs in the spleen and the serum hemagglutinating titer were partly restored in mice treated with both cyclophosphamide and ginsenoside Rg1 as compared to control mice treated with cyclophosphamide only. Thus, the number of PFCs was increased by 165.0, 79.7, 100.0 and 151.0 percent, respectively, at 3, 5, 7 and 10 days after the immunization. The rise in the hemagglutinating titer was 16-fold on the 7th day and 4-fold on the 10th and 15th day after the immunization in the ginsenoside Rg1-treated mice.

2. Effect of ginsenoside Rg1 on antigen-reactive T-cells: The influence of ginsenoside Rg1 on T-cell proliferation in vivo was determined by measuring the number of rosette-forming cells. The data presented on Table 3 show that in vivo administration of ginsenoside Rg1 increased the number of antigen-reactive T-lymphocytes by 135.0, 27.8 and 44.2 percent, respectively, at 3, 5 and 7 days after the immunization. This effect was even more pronounced in mice pretreated with cyclophosphamide, and it persisted up to the 15th day after the immunization (P<0.05).

3. Effect of ginsenoside Rg1 on cell-mediated immune response: The changes in the cell-mediated immune reaction after

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Table 1. Plaque-forming cell response to SRBC antigen in mice treated with ginsenoside Rg1 and pretreated with cyclophosphamide and treated with ginsenoside Rg1.

| Days after immunization | 3  | 5  | 7  | 10 | 15 |
|-------------------------|----|----|----|----|----|
| Treatment               |    |    |    |    |    |
| Control (PBS only)      | 150±25 | 173±12 | 226±16 | 107±9 | 65±7 |
| Ginsenoside Rg1         | 194±16 | 251±10* | 304±28* | 228±42* | 76±7 |
| Control (cyclophosphamide only) | 40±9 | 69±12 | 74±12 | 37±8 | 29±8 |
| Cyclophosphamide        | 106±26* | 124±16* | 148±19* | 93±3* | 38±9 |
| Ginsenoside Rg1         |    |    |    |    |    |

*Pooled from 5 mice. *Statistically significant difference from the control at P<0.05.

Table 2. Hemagglutinating antibody titer to SRBC antigen in mice treated with ginsenoside Rg1 and in mice pretreated with cyclophosphamide and treated with ginsenoside Rg1.

| Days after immunization | 3  | 5  | 7  | 10 | 15  |
|-------------------------|----|----|----|----|-----|
| Treatment               |    |    |    |    |    |
| Control (PBS only)      | 4  | 128 | 512 | 1024 | 1024 |
| Ginsenoside Rg1         | 4  | 256 | 4096 | 2048 | 2048 |
| Control (cyclophosphamide only) | 4 | 4 | 8 | 32 | 64 |
| Cyclophosphamide        | 4  | 4 | 128 | 128 | 256 |
| Ginsenoside Rg1         |    |    |    |    |    |

*Pooled from 5 mice.
ginsenoside Rg₁ treatment were measured by the differences in the weight of the popliteal lymph nodes 24 hr after the antigen challenge. Ginsenoside Rg₁ enhanced the cell-mediated immune response: the popliteal lymph node index was 2.27±0.10 in the treated mice as compared to 1.50±0.12 in the control mice (P<0.001).

### Table 3. Antigen-reactive T-cell response to SRBC in mice treated with ginsenoside Rg₁ and in mice pretreated with cyclophosphamide and treated with ginsenoside Rg₁.

| Days after immunization | Treatment                              | Antigen-reactive T-cells per 10⁷ splenocytes³ |
|-------------------------|----------------------------------------|---------------------------------------------|
|                         | Control (PBS only)                      | 5166±63                                     |
|                         | Ginsenoside Rg₁                         | 12166±75*                                   |
|                         | Control (cyclophosphamide only)         | –                                           |
|                         | Cyclophosphamide                       | 4666±90                                     |
|                         | Ginsenoside Rg₁                         | 4666±90                                     |

³Pooled from 5 mice. *Statistically significant difference from the controls at P<0.05.

### Table 4. T-helper cell response to SRBC antigen in mice treated with ginsenoside Rg₁ and in mice pretreated with cyclophosphamide and treated with ginsenoside Rg₁.

| Days after immunization | Treatment                              | T-helper cell percentage³ |
|-------------------------|----------------------------------------|---------------------------|
|                         | Control (PBS only)                      | 62.9±1.0                  |
|                         | Ginsenoside Rg₁                         | 83.6±1.2*                 |
|                         | Control (cyclophosphamide only)         | 26.1±1.4                  |
|                         | Cyclophosphamide                       | 48.2±1.7*                 |
|                         | Ginsenoside Rg₁                         | 48.2±1.7*                 |

³Expressed as percentage of the whole T-cell number *Statistically significant difference from the controls at P<0.05.

4. Effect of ginsenoside Rg₁ on T-helper cell number: Ginsenoside Rg₁ also changed the number of T-helper cells. Thus, the percentage of T-helper cells (with respect to the whole T-cell number) was increased in the treated mice as compared to 1.50±0.12 in the control mice (P<0.001).

5. Effect of ginsenoside Rg₁ on natural killer activity: The natural killer activity of the splenocytes was measured using chick erythrocytes as target cells 5 and 10 days after ginsenoside Rg₁ treatment. There was an approximately threefold increase in the natural killer activity: the specific lysis was 57.8±1.6 percent in the treated mice as compared to 21.6±1.9 percent in the controls (P<0.001). On the 10th day after the application of ginsenoside Rg₁, its effect on the natural killer activity was negligible.

6. Effect of ginsenoside Rg₁ on IL-1 production: The in vitro effect of ginsenoside Rg₁ on IL-1 production by peritoneal macrophages (pMΦ) at the doses of 25 and 50 µg/ml on IL-1 production is shown in Fig. 2. The lower dose of ginsenoside Rg₁ (25 µg/ml) induced a greater production of IL-1 than the higher dose (50 µg/ml). It is interesting to note that the augmentation of IL-1 production caused by the lower dose of Rg₁ was similar to the stimulation caused by the LPS
of E. coli. Ginsenoside Rg₁ also exerted direct mitogenic effect when applied in vitro to microcultures of thymus cells only. Again this effect was greater with the lower dose (25 μg/ml), while the higher dose of Rg₁ (50 μg/ml) had no significant mitogenic effect on thymocytes.

Discussion

The present results indicated that ginsenoside Rg₁ modulated both the humoral and cell-mediated immune response in mice. In vivo administration of Rg₁ caused an increase in the number of PFCs and in the titers of circulating antibodies as well as in the number antigen-reactive T-cells. The observed effect of ginsenoside Rg₁ on the humoral and cell-mediated immune response was moderate. The increase in the number of antigen-reactive T-cells correlated with the increase of popliteal lymph node indexes in the treated mice since it is well-known that T-cell activation triggers the mononuclear infiltration of the cell-mediated immune response.

Ginsenoside Rg₁ also changed the ratio of the number of T-helper cells to the whole number of T-cells. The increased activity of the natural killer cells, which is known to respond to lymphokines such as interleukin-2 (IL-2), released by T-helpers responding to antigen (16) was consistent with the increased number of T-helper cells. These results are in agreement with the data about inhibition of sarcoma cell growth caused by
this triterpenoid saponin and are consistent with some of its beneficial effects in the treatment of gastric cancer (17).

Although ginsenoside Rg₁, did not modulate the phagocytic activity of macrophages and polymorphonuclear cells (data not shown), its in vitro application augmented IL-1 production by peritoneal macrophages. It should be pointed out that Rg₁ was effective at the lower dose (25 μg/ml) while the higher dose induced insignificant changes in IL-1 production. The lower dose of this substance also exerted a direct mitogenic effect on microcultures of thymus cells, while the higher dose was ineffective. These results favor the view of Wybran (18) that most of the immunomodulators in higher doses cause immunosuppression, while in lower doses, it lead to immunostimulation.

Taken together, our in vivo and in vitro findings suggest that ginsenoside Rg₁ does not modulate specifically a single component of the immune system but influences more than one step of the immune reactions. It might be further speculated that ginsenoside Rg₁ possesses the ability to restore the cyclophosphamide-impaired humoral and cell mediated immune responses through activation of IL-1 production by macrophages and that it possibly induced production of other lymphokines as well. It has recently been shown that IL-1, whose production is increased by ginsenoside Rg₁, is also able to increase the in vivo antigen specific T-cell proliferation (19) and the serum antibody response (20). Although our data are only indirect, it cannot be excluded that ginsenoside Rg₁ also stimulates IL-2 production by T-cells since it has a direct mitogenic effect on thymocytes and its in vivo administration causes an increase in the T-helper subpopulation.

However, the precise mechanism of the immunomodulatory action of ginsenoside Rg₁ is not clear as yet. These results only show that ginsenoside Rg₁ from Panax ginseng in the mode of application used in the present experiments exerts a potentiating effect on the immune system, probably through activation of some steps in the macrophages– T-cell lymphokine cascade reaction. Further studies are required to establish the exact mechanism of its action and to evaluate the modulatory activity of the other components of Panax ginseng on the immune system.

References
1 Saito, H.: Effects of ginsenoside Rb₁ and Rg₁ on memory and learning. International Ginseng Seminar, Tokyo, April 1st, p. 27–30 (1989)
2 Petkov, V.D. and Staneva, D.: Der Einfluss eines Ginseng Extraktes auf die Funktionen Nebennierenrinde. Arzneimittel Forschung 13, 1078–1083 (1963)
3 Petkov, V.D. and Mosharov, A.H.: Effects of standardized ginseng extract on learning, memory and physical capabilities. Am. J. Chin. Med. 15, 19–29 (1987)
4 Lazarova, M.B., Mosharov, A.H., Petkov, V.D., Markovska, V.L. and Petkov, V.V.: Effect of piracetam and of standardized ginseng extract on the electroconvulsive shock-induced memory disturbances in "step-down" passive avoidance. Acta Physiol. Pharmacol. Bulg. 13, 11–17 (1987)
5 Petkov, V.D. and Mosharov, A.H.: Age and individual-related specificities in the effects of standardized ginseng extract on learning and memory (experiments on rats). Phytother. Res. 1, 80–84 (1987)
6 Singh, V.K., George, C.X., Singh, N., Agarwal, S.S. and Gupta, B.M.: Combined treatment of mice with Panax ginseng extract and interferon inducer. Planta Med. 47, 234–236 (1983)
7 Singh, V.K., Agarwal, S.S. and Gupta, B.M.: Immunomodulatory activity of Panax ginseng extract. Planta Med. 50, 462–465 (1984)
8 Shibata, S.: Some recent studies on ginseng saponins. International Gerontological Symposium, Singapore, February 14–15, p. 183–197 (1977)
9 Shibata, S.: Some chemical studies on ginseng. Symposium of Gerontology, Lugano, April 9th, p. 124–131 (1975)
10 Wagner, H.: Immunostimulants from higher plants (recent advances). In Biologically Active Natural Products. Edited by Hostettman, K. and Lea, P.J., p. 127–141, Clarendon Press, Oxford (1987)
11 Cunningham, A.J. and Szenberg, A.: Further improvement in the plaque technique for detecting single antibody forming cells. Immunology 11, 599–600 (1966)
12 Mendes, N.F., Tolmat, M.E., Silvestra, N.P., Gilberstein, R.B. and Metzgar, R.S.: Technical aspects of the rosette tests used to detect human complement receptor (B) and sheep erythrocyte
binding (T) lymphocytes. J. Immunol. 11, 860-867 (1973)

13 Gleichmann, H.: Studies on the mechanism of drug sensitization: T-cell dependent popliteal lymph node reaction to diphenylhydantoin. Clin. Immunol. Immunopathol. 180, 203-211 (1981)

14 Shore, H., Dosch, H.-M. and Geltand, E.W.: Induction and separation of antigen-dependent helper and T suppressor cells in man. Nature 274, 586-587 (1978)

15 Mizel, S.B.: Production and quantitation of lymphocyte-activating factor (interleukin-1). In Manual of Macrophage Methodology, Edited by Herscowitz, H.B., Holden, H.T., Belanti, J.A. and Ghaffar, A., p. 407-409. Marcel Dekker, New York (1981)

16 Pope, B.L.: Immunopharmacology: a new frontier. Can. J. Physiol. Pharmacol. 67, 537-545 (1989)

17 Hiller, K.: New results on the structure and biologic activity of triterpenoid saponins. In Biologically Active Natural Products. Edited by Hostettman, K. and Lea, P.J., p. 167-184. Clarendon Press, Oxford (1987)

18 Wybran, J.: Immunomodulation. Overview. Current Opinion in Immunology 1, 251-252 (1988)

19 Durum, S.K., Higuchi, C. and Ron, Y.: Accessory cells and T cell activation. The relationship between two components of macrophages accessory function: I-A and IL-1. Immunobiology 168, 213-218 (1984)

20 Staruch, M.J. and Wood, D.D.: The adjuvanticity of interleukin 1 in vivo. J. Immunol. 130, 2191-2196 (1983)