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IMMUNOSTIMULATING EFFECTS OF ACIDIC POLYSACCHARIDES EXTRACT OF *PANAX GINSENG* ON MACROPHAGE FUNCTION

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ABSTRACT

The root of *Panax ginseng* C. A. Meyer is one of the most popular natural tonics in oriental countries. In this study, we have isolated polysaccharide fraction of *Panax ginseng* (ginsan) and examined its effect on the function of murine peritoneal macrophages. When macrophages were treated with ginsan, cytotoxic activity against B16 melanoma cells was significantly induced. In addition, the levels of cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6 and lnterferon-γ (IFN-γ) were increased and the production of reactive oxygen/nitrogen components such as nitric oxide (NO) and hydrogen peroxide (H_2O_2) was enhanced. Moreover, phagocytic activity was induced in ginsan-treated macrophages compared to the control. The expression of CD14 and l-A^b on murine peritoneal macrophages was

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increased by the treatment with ginsan, while the expression of CD11b was decreased. Taken together, these results suggest that ginsan has an immunopotentiating effects on macrophages and these abilities could be used clinically for the treatment of diseases such as cancer.

INTRODUCTION

Macrophages have been known to play an essential role in host defense mechanism. They show extrinsic and intrinsic resistances to viral infection.[1,2] The cytotoxic activities of macrophages are also involved in the resistance to tumors.[3] Macrophages can be activated by a variety of agents and some of these have been shown to increase tumoricidal effects when administered in vivo. Treatment of mice with *Propionibacterium acne* has increased resistance to tumor cells.[4] This increased resistance to tumors may be partially due to increased macrophage cytotoxic activity.

The root of *Panax ginseng* C.A. Meyer (Araliaceae) is one of the most popular natural tonics which has been used in Asian countries. The water extracts of ginseng showed antitumor activity against some kinds of tumor cells in mice[5] and inhibited the incidence of lung tumors by a wide range of carcinogens.[6] In epidemiologic studies, ginseng intake reduced the incidence of human cancers.[7] However, the active substance and its mechanism of action have not been elucidated yet. In the previous study, we isolated an antineoplastic immunostimulator from ginseng, acidic polysaccharide named ginsan,[8] and studied its effect on production of cytokine from LAK cells and metastasis inhibition of tumor cells.[9] In addition, it has been proposed that cytokines produced from splenocytes could be involved in the generation of cytotoxic cells by ginsan.[9] However, it has not been yet defined how ginsan activates macrophages and which factors of macrophage are involved in cytotoxicity to tumor cells. In order to gain further insight into the effect of ginsan on macrophage activities, we tested various functions of ginsan-treated macrophages and suggested how ginsan could prevent the tumor growth by increasing the tumoricidal activity of murine peritoneal macrophages.

MATERIAL AND METHODS

Mouse and Chemicals

Six to eight weeks old male C57BL/6 mice were obtained form Charles River Breeding Laboratories (Atsugi, Japan). Unless otherwise indicated, all chemicals were purchased from Sigma Chemical Co. (St. Louis, USA). RPMI-1640 medium and fetal bovine serum (FBS) were purchased from GIBCO (Grand Island, USA). FITC-labelled anti-CD14, PE-labelled anti-

CD11b, PE-labelled anti-I-A^b antibodies and recombinant murine TNF-α were purchased from PharMingen (San Diego, USA). FITC-labelled goat animouse immunoglobulin was purchased from Jackson Immuno Research Laboratories (WestGrove, PA). All tissue culture reagents, the thioglycollate broth and acidic polysaccharide (ginsan) were assayed for endotoxin contamination by the Limulus lysate test (E-Toxate, Sigma) and found to be less than 10 pg/mL.

Preparation of Ginsan

Acidic polysaccharide ginsan, M.W. of 150,000, composed of 3.7% protein, 47.1% hexose (glucose and galactose) and 43.1% uronic acid (galacturonic acid), was purified from the ethanol insoluble fraction of ginseng water extract (*Panax ginseng* C.A. Meyer, Araliaceae).[9]

Isolation of Inflammatory Peritoneal Macrophages

Thioglycollate-elicited peritoneal exudate cell were obtained from C57BL/6 mice following intraperitoneal injection of 1 mL thioglycollate and lavage of the peritoneal cavity with 5 mL of medium after 3–4 days. The cells were washed twice with Dulbecco's phosphate buffered saline (D-PBS) (GIBCO, Grand Island, USA) and resuspended in RPMI-1640 containing 10% FBS and 2% penicillin/streptomycin.[10] Macrophages were isolated from peritoneal exudate cells as described by Klimetzek and Remold.[11] Peritoneal exudate cells were seeded at densities of $5-6\times10^5$ cells/cm^2 on teflon-coated petri dishes (100×15 mm) and the macrophages were allowed to adhere for 2 h in 5% CO_2 humidified atmosphere. Teflon-coated petri dishes were prepared by spraying with aerosolized Teflon (Fisher Scientific, Pittsburgh, PA) and sterilized with ultraviolet light for 3 h. After 2 h, the non-adherent cells were removed by washing the dishes twice with 10 mL pre-warmed Dulbecco's phosphate buffered solution (D-PBS) (GIBCO, Grand Island, NY, USA). The dishes were incubated for 10 min at 4°C. The supernatants were carefully removed and discarded and the dishes were washed once with D-PBS. 15 mL cold D-PBS containing 1.5% FBS was added and followed by 0.3 mL PBS containing 0.1 M EDTA (pH 7.0). The dishes were incubated for 15 min at room temperature and the macrophage was removed by rinsing ten times using a 10 mL syringe. The viability of the detached cells was assessed by trypan blue exclusion and the proportion of macrophages was determined by a fluorescence microscope examination of acridine orange stained cells. The cell preparations were >95% viable and contained >95% macrophages.

Treatment of Peritoneal Macrophages with Ginsan

Peritoneal macrophages were seeded at a concentration of 1×10^5 cells/mL in 96 wells tissue culture plates (Nunc, Denmark). Non-adherent cells were discarded and the culture plates washed once with D-PBS. The macrophages were treated with various doses of ginsan. These plates were incubated for 24 h in 5% CO_2 humidified atmosphere. The necessary assay was performed to measure the effect of ginsan on macrophage function.

Macrophage-mediated Cytotoxicity

The determination of macrophage-mediated cytotoxicity was assessed by modification of the technique described previously.[12] Briefly, macrophages (1.0×10^5 cells/well) were first incubated in either medium alone or in medium supplemented with ginsan for 24 h in 96-well plates. Macrophages were washed with RPMI-FBS to remove ginsan and then co-incubated with B16 melanoma cells (1.0×10^4/wells; an initial effector: target cell ratio of 10:1) at 37°C in a 5% CO_2 incubator. Cell density was then assessed by incubating the cells with 25 μg/mL MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide] for another 4 h. Formazan produced was dissolved in dimethyl sulfoxide and the optical density of each well at 540 nm was determined using a Molecular device microplate reader (Menlo Park, CA). Cytolytic activity is expressed as the percentage of tumor cytotoxicity where

$$\% \text{ Cytotoxicity} = \left\{1 - \frac{\text{O.D. of } [(\text{target} + \text{macrophages}) - \text{macrophages}]}{\text{O.D. of target (nontreated)}}\right\} \times 100$$

ELISA Assay of IL-1β, IL-6, TNF-α, and IFN-γ

Accumulation of IL-1β, IL-6, TNF-α and IFN-γ in culture supernatants was determined using each ELISA kit (PharMingen, San Diego, CA) according to the manufacturer's instructions.

Determination of NO Production

The production of nitrite in supernatants was measured by nitric oxide detector (Antek Model 7020, Houston, TX).

Determination of H_2O_2 Production

The secretion of hydrogen peroxide in culture supernatants was fluorimetrically measured by the horseradish peroxidase-catalysed oxidation of fluorescent scopoletin to a nonfluorescent product, as described in detail.[13] The 460 nm emission from reduced scopoletin, when excited by light at 350 nm, is extinguished when scopoletin is oxidized by H_2O_2 in the presence of horseradish peroxidase. Under assay conditions, the loss of fluorescence was proportional to the concentration of H_2O_2.

Assay of Phagocytic Activity

The phagocytotic activity was measured using the assay system described previously.[14] Macrophages were treated with indicated concentration of ginsan for 24 h and then washed with RPMI1640 to remove ginsan. Cells were incubated with 5×10^6 particle of zymosan and 600 μg/mL of NBT. After 1 h incubation, plates were centrifuged at 4°C for stopping ingestion of zymosan and supernatant was removed by flipping. The optical density of the reduction product of NBT, a purple insoluble formazan, was determined at 540 nm using a Molecular device microplate reader. It was not required to solubilize the formazan before taking the measurement of absorbance.

Assay of Surface Antigen Expression

The cell-surface expression of CD14, CD11b and I-A^b was determined by staining 5×10^5 cells suspended in PBS containing 10% FBS with a saturating concentration of FITC-conjugated or PE-conjugated monoclonal antibodies to cell-surface antigens along with the appropriate isotype control for 30 min on ice. Cells were washed twice with PBS containing 10% FBS, and bound antibody was detected with a FACScan flow cytometer (Beckton & Dickinson, San Jose, CA).

Statistical Analysis

Each data were given as means ± S.E.M. Statistical difference between groups was determined by one-way analysis of variance (ANOVA) and significant values were represented by an asterisk ($*p < 0.05$).

RESULTS

Inductive Effect of Macrophage-mediated Cytotoxicity

To examine whether ginsan-treated macrophages are able to kill B16 melanoma cells which were sensitive to TNF-α or NO, macrophages were treated with various doses of ginsan for 24 h and subsequently co-incubated with B16 melanoma cell for 16 h. As shown in Fig. 1, ginsan (1–100 μg/mL) increased the cytotoxicity by macrophages in a concentration-dependent manner. Ginsan did not affect the viability of macrophages, but concentrations greater than 100 μg/mL were cytotoxic to cells (data not shown). In addition, the effects of ginsan were not due to contamination by endotoxin, which was $< 0.3 \times 10^{-3}$ international unit per microgram of ginsan as assessed by the limulus test.

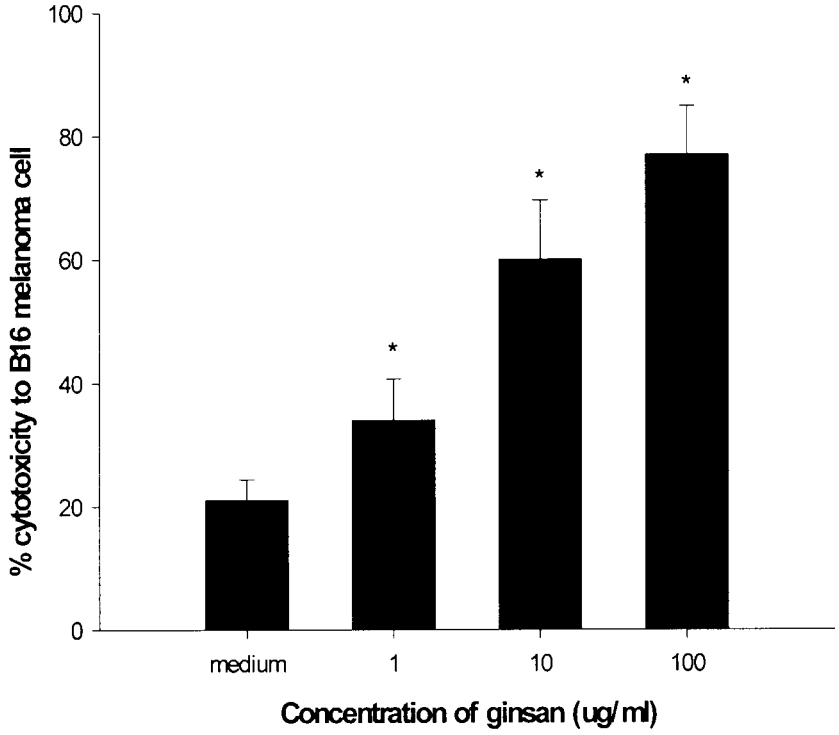

Figure 1. Tumoricidal activities of ginsan-treated murine peritoneal macrophages against B16 melanoma cells. Macrophages were treated with various doses of ginsan for 24 h. Antitumor activity was determined as described in materials and methods at an initial effector/target ratio of 10:1. The results are mean ± S.E.M of three independent experiments. *$p < 0.05$; significantly different from control (no treatment).

Production of Cytokines by Macrophages with Ginsan

We examined the effect of ginsan on the secretion of TNF-α, IL-1β, IL-6, and IFN-γ by macrophages. Murine peritoneal macrophages were treated with 1, 10, and 100 μg/mL ginsan for 24 h and culture supernatants were used for the measurement of each cytokine. Production of TNF-α at 100 μg/mL was significantly higher than that in the medium (Fig. 2a). IL-1β, IL-6, and IFN-γ were also significantly increased by exposure to ginsan at 10 and 100 μg/mL (Fig. 2b, 2c, 2d) as compared to control (medium alone). These data indicate that ginsan is a potent inducer of cytokine production from macrophages in vitro.

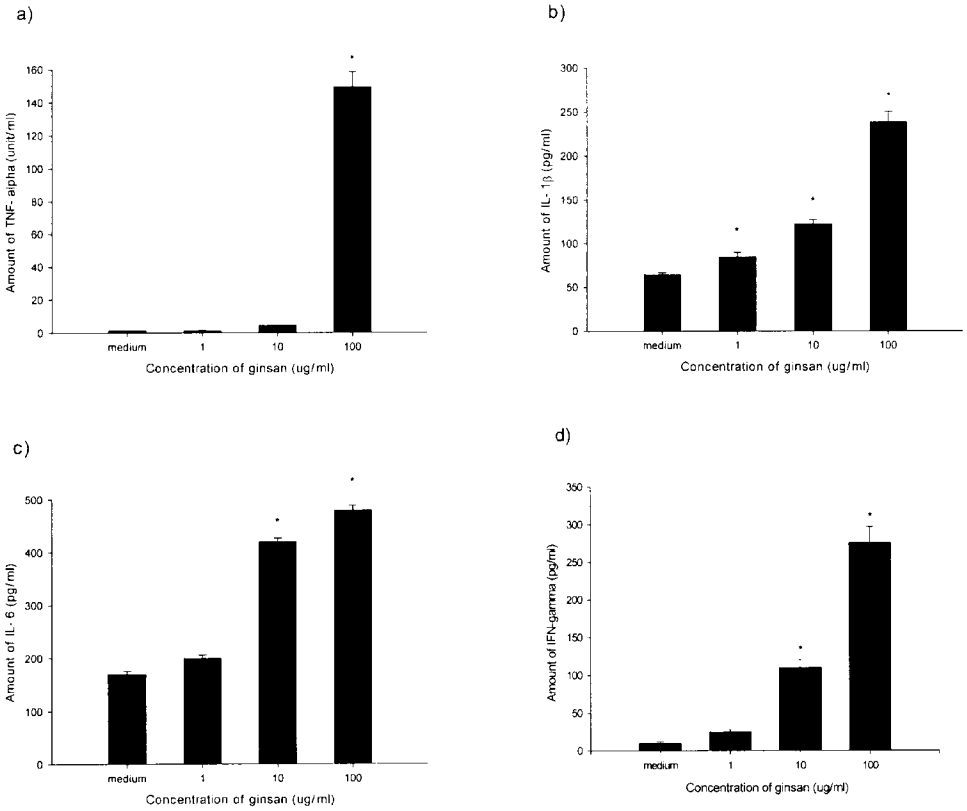

Figure 2. TNF-α (a), IL-1 (b), IL-6 (c), and IFN-γ (d) production by peritoneal macrophages stimulated by ginsan. Macrophages were treated with ginsan for 24 h. Culture supernatants were collected and the levels of cytokines were measured by ELISA. The results shown are the mean ± S.E.M of three independent experiments. $*p < 0.05$; significantly different from control (no treatment).

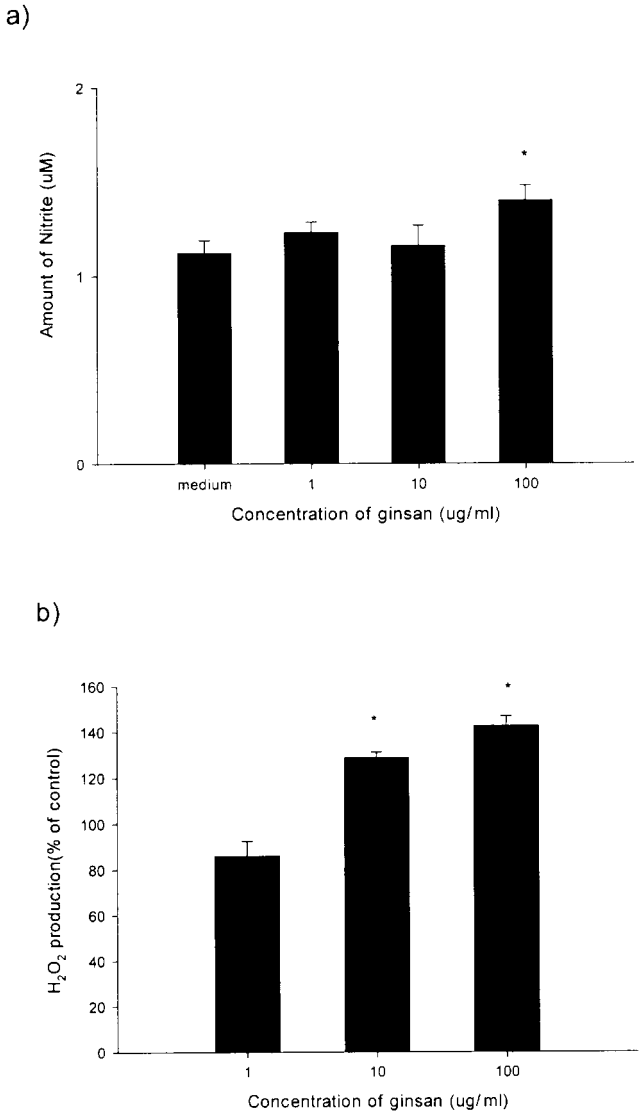

Figure 3. Nitrite (a) and H_2O_2 (b) production from peritoneal macrophages stimulated with ginsan. Macrophages were treated with ginsan for 24 h. Culture supernatants were collected and the levels of nitrite and H_2O_2 were measured as described in materials and methods. The results are mean $\pm$ S.E.M of three independent experiments. $^*p < 0.05$; significantly different from control (no treatment).

Production of NO and H_2O_2 by Macrophages with Ginsan

To determine the effect of ginsan on the production of NO and H_2O_2 by murine peritoneal macrophages, macrophages were treated with various doses of ginsan for 24 h. Culture supernatant was tested as previously

described method. As shown in Fig. 3a, the production of NO was not greatly increased. NO production in only 100 μg/mL of the ginsan-treated group was slightly significant in comparison with that of the control group. However, the production of NO was not only persisted but also more increased at dosage levels tested after 24 h (data not shown). When macrophages were treated with 10 and 100 μg/mL of ginsan, H_2O_2 production was increased to about 30% or 40% as compared to that in untreated cells, respectively (Fig. 3b). Since NO and H_2O_2 have been implicated in macrophage cytotoxicity, our results imply that NO and H_2O_2 are involved in tumoricidal activities of stimulated macrophages.

Enhancement of Phagocytic Activity

Peritoneal macrophages treated with various doses of ginsan for 24 h were assessed for their abilities to ingest zymosan particles. As shown in Fig. 4, phagocytosis is not evident in cells from medium-treated cultures, while treatment of cells with ginsan results in a significant increase in the macrophage function tested. In addition, we have observed the NBT reduction that is correlated to phagocytic activity quantitatively (data not shown). These data provide further evidence that ginsan is able to induce macrophage functional activity.

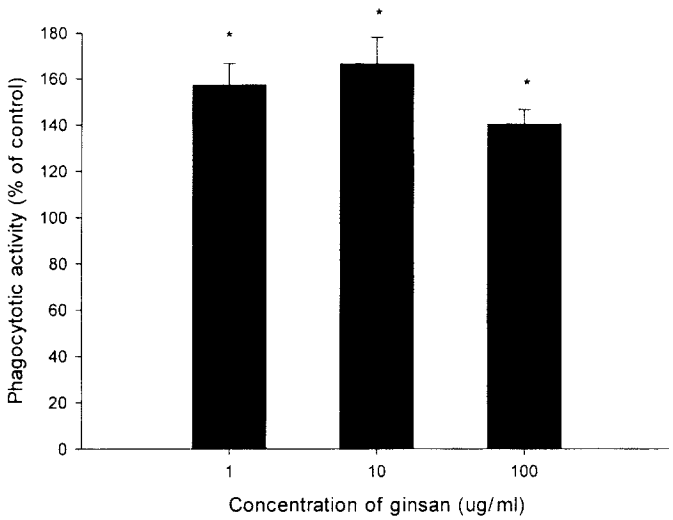

Figure 4. Effect of ginsan on the phagocytosis of murine peritoneal macrophages. Macrophages were treated with ginsan for 24 h. Macrophages were then incubated with 5×10^6 particle of zymosan and 600 μg/mL of NBT. Phagocytosis was measured as OD 540 nm. The results shown are the mean ± S.E.M of three independent experiments. $*p < 0.05$; significantly different from control (no treatment).

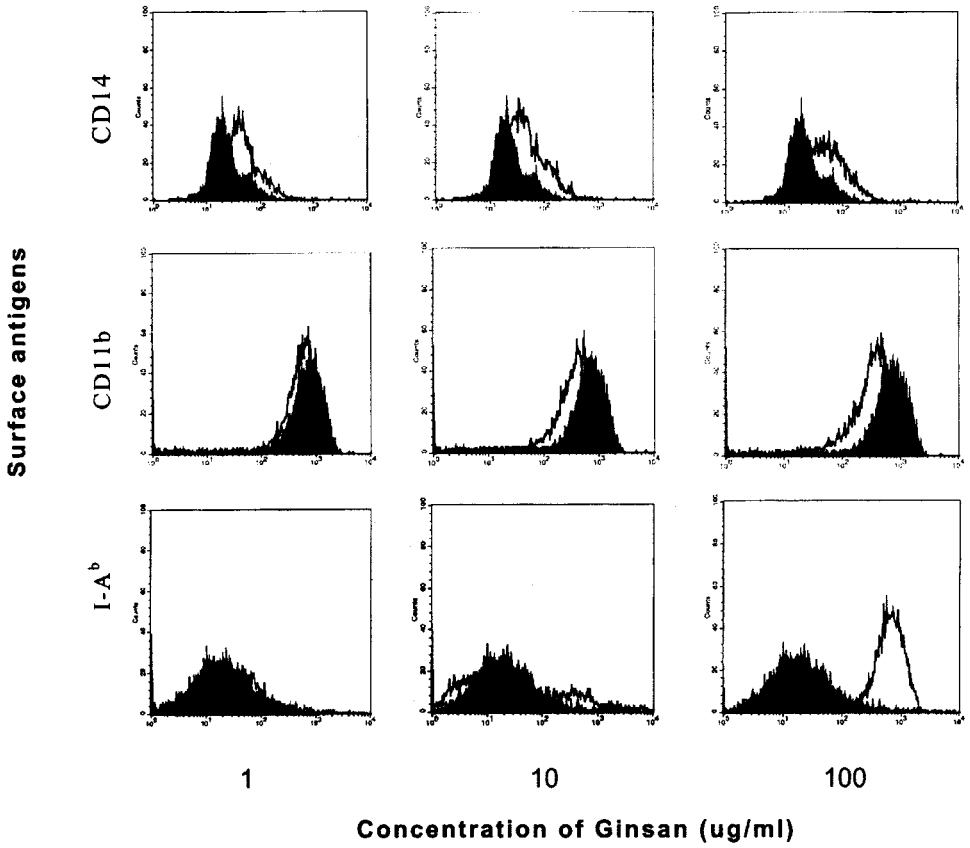

Figure 5. Surface expression of CD14, I-2^b, and CD11b on macrophages treated with ginsan. Macrophages were treated with ginsan for 24 h. Cells were detached and stained with each fluorescence-labelled antibody (open peaks) or isotype control antibody (filled peaks) as described in material and methods. This is a representative staining pattern of three experiments.

Modulation of Cell Surface Antigens on Macrophages

Macrophages treated with ginsan were examined for the expression of cell surface antigens CD11b, CD14 and I-A^b to further analyze the activation status of these cells. As shown in Fig. 5, the expression of CD14 (ligand of LPS-binding protein) and I-A^b (MHC class II molecule) was increased by ginsan. In contrast, the constitutive levels of CD11b (Mac-1, adhesion molecule) appeared to be reduced at high concentrations, implicating that the level of surface antigen expression is altered by ginsan treatment of macrophages. In addition, these results suggest that increased expression of

CD14 and I-A^b is correlated with many ginsan-induced responses in macrophages.

DISCUSSION

It is well known that macrophages play a pivotal role in tumor immunity. They directly kill tumor cells by secreting cytotoxic molecules or indirectly by recruitment and activation of other immune cells in relation to tumor suppression. Thus, effector molecules produced by activated macrophages help to eliminate abnormal tumor cells.[15] In this study, we investigated the potential of acidic polysaccharide of *Panax ginseng*, ginsan, to activate murine peritoneal macrophages as determined by cytotoxicity, phagocytic activity and production of secretory molecules. We also determined whether the expression of cell surface antigens can be modulated by ginsan. Our results demonstrated that ginsan-treated macrophages induced tumoricidal activity, increase phagocytic activity and exhibit the capacity to produce cytokines and cytotoxic molecules. Moreover, ginsan is able to enhance the expression of CD14 and 1-A^b, whereas the expression of CD11b was slightly decreased by ginsan treatment.

In response to a variety of stimuli macrophages produce numberous secretory components including monokines, chemokines, reactive oxygen intermediates and reactive nitrogen intermediates. Reactive oxygen/nitrogen intermediates have been implicated as mediators of tumoricidal activity.[16,17] It has been also known that cytokines are involved in tumoricidal activity of macrophages.[18] In the present experiments, our results suggest that nitric oxide and H_2O_2 induced by ginsan are the effector molecules involved in macrophages-mediated tumor cytostasis. In addition, the present data show that ginsan-treated macrophages were able to induce the production of IL-1β, IL-6, TNF-α, and IFN-γ. These results suggest that the immunostimulatory activity of ginsan may be mediated through upregulation of secretory molecules in macrophages and ginsan plays a role in triggering the activation of macrophages. Recently, Chen, et al. showed that cytokine combination acts synergistically to activate macrophages and IFN-γ plays an important role in this synergistic effect.[19] In addition, Boehm et al. demonstrated that the production of IFN-γ was not only directly auto-regulated by intracellular signaling but also indirectly controlled by TNF-α.[20] We have also found that the production of IFN-γ was totally or partially blocked by treatment of anti-TNF-α antibodies or iNOS inhibitor, NMMA (data not shown). Based on these findings it is conceivable that TNF-α and NO affect IFN-γ production in ginsan-treated macrophages and IFN-γ synergizes with other secretory compounds to activate cells via autocrine or paracrine mode. However, further studies will be needed to elucidate the cytokine interaction of macrophages that are exposed to various stimuli.

A large number of cell surface molecules are present on the surface of macrophages and are largely the means by which this cell type interacts with its environment. It has been reported that expression of CD14 antigen, known as LPS receptors, is positively up-regulated by LPS[21,22] and this signal participates in release of cytokines, including TNF-α.[23,24] Our data show that the expression of CD14 is significantly increased in ginsan-treated macrophages, suggesting that the stimulatory effects of ginsan on macrophages may occur via CD14-dependent pathway and the cell activation pathway of ginsan is similar to that of LPS. Moreover, since ginsan does not have lipid moiety, such as lipid A, which bind to LPS-binding protein (LBP), it is likely that ginsan can activate macrophages by directly binding CD14 via LBP-independent pathway. However, one cannot rule out the possibility that ginsan stimulates macrophages via CD14-independent pathway. Recently, it has been reported that CD14 tethers membrane by glycosyl-phosphatidyl-inositol (GPI) anchor and innate immune response requires Toll-like receptor (TLR) as signaling receptor for transferring LPS signals.[25–28] Thus, these findings imply that ginsan can form a complex with TLR to tranduce extracellular stimuli into macrophages in CD14-independent pathway. We are currently investigating this possibility.

It has been known that up-regulation of MHC class II molecules is a principal mechanism of action on which enhances the interaction between macrophages and T cells.[15] The present data show that ginsan treatment increased the background level expression of MHC class II molecules, I-A^b, suggesting that ginsan stimulates macrophages to activate other immune cells via cell-to-cell communication resulting in the modulation of various immune responses. In contrast, the expression of CD11b is decreased in ginsan-treated macrophages. Since the expression of adhesion proteins such as CD11b plays a role in migration in vivo,[29] our data indicate that ginsan may not affect the migration of macrophages.

In conclusion, our results show that ginsan, acidic polysaccharides extracted from *Panax ginseng*, is able to stimulate the production of secretory molecules, phagocytic activity and tumoricidal activity of macrophages. Furthermore, CD14 and MHC class II modulation were affected. These immunomodulating activities of ginsan could be used clinically for the modulation of immune response. Further studies will be required to clarify how this modulation occurs and to what extent it occurs in vivo.

REFERENCES

1. Morahan, P.S. In *Immunobiology of Herpes Simplex Virus Infection*; Rouse, B.T., Lopez, C., Eds.; CRC Press: Boca Raton, 1984; 71.
2. Morahan, P.S.; Kern, E.R.; Glasgow, L.A. Viruses and the Versatile Macrophage. Br. Med. Bull. **1985**, *410*, 150.

3. Cohn, Z.A. Activation of Mononuclear Phagocytes: Fact, Fancy, and Future. J. Immunol. **1978**, *121*, 813.
4. Campbell, M.W.; Scholley, M.M.; Miller, G.A. Macrophage Heterogeneity in Tumor Resistance: Cytostatic and Cytotoxic Activity of Corynebacterium parvum-activated and Proteose Peptone-elicited Rat Macrophages Against Moloney Sarcoma Tumor Cells. Cell. Immunol. **1980**, *50*, 153.
5. Lee, K.D.; Huemer, R.P. Antitumoral Activity of Panax ginseng Extracts. Jpn. J. Pharmacol. **1971**, *21*, 299.
6. Yun, T.K.; Yun, Y.S.; Han, I.W. Anticarcinogenic Effect of Long-term Oral Administration of Red Ginseng on Newborn Mice Exposed to Various Chemical Carcinogens. Cancer Detect. Prev. **1983**, *6*, 515.
7. Yun, T.K.; Choi, S.Y. A Case-control Study of Ginseng Intake and Cancer. Int. J. Epidemiol. **1990**, *19*, 871.
8. Yun, Y.S.; Lee, Y.S.; Jo, S.K.; Jung, I.S. Inhibition of Autochthonous Tumor by Ethanol Insoluble Fraction from Panax ginseng as an Immunomodulator. Planta. Med. **1993**, *59*, 521.
9. Yun, Y.S.; Kim, K.H.; Lee, Y.S.; Jung, I.S.; Park, S.Y.; Chung, H.Y.; Lee, I.R. Acidic Polysaccharide from Panax ginseng, Ginsan, Induces Th1 Cell and Macrophage Cytokines and Generates LAK Cells in Synergy with rIL-2. Planta. Med. **1998**, *64*, 110.
10. Moon, E.Y.; Pyo, S. Inhibition of Various Functions in Murine Peritoneal Macrophages by Aflatoxin B1 Inhibits Exposure in vivo. Int. J. Immunopharmaco. **1999**, *21*, 47.
11. Klimetzek, V.; Remold, H.G. The Murine Bone Marrow Macrophage, a Sensitive Indicator Cell for Murine Migration Inhibitory Factor and a New Method for Their Harvest. Cell. Immunol. **1980**, *53*, 257.
12. Flick, D.A.; Gifford, G.E. Comparison of in vitro Cell Cytotoxicity Assays for Tumor Necrosis Factor. J. Immunol. **1984**, *68*, 167.
13. Nathan, C.F.; Root, R.K. Hydrogen Peroxide Release from Mouse Peritoneal Macrophages: Dependence on Sequential Activation and Triggering. J. Exp. Med. **1977**, *1461*, 1648.
14. Okimura, T.; Ogawa, M.; Yamauchi, T. Stress and Immune Responses. III. Effect of Resistant Stress on Delayed Type Hypersensitivity (DTH) Response, Natural Killer (NK) Activity and Phagocytosis in Mice. Jpn. J. Pharmacol. **1986**, *41*, 229.
15. Rees, R.C.; Parry, H. In *The Macrophage*; Lewis, C.E., McGee, J.O.D., Eds.; Oxford University Press: Oxford, 1992; 315.
16. Mavier, P.; Edgington, T.S. Human Monocyte-Mediated Tumor Cytotoxicity. Demonstration of an Oxygen Dependent Myeloperoxidase-independent Mechanism. J. Immunol. **1984**, *132*, 1980.
17. Hibbs, J.B.; Taintor, R.R.; Vavrin, Z. Macrophage Cytotoxicity: Role for L-Arginine Deiminase and Imino Nitrogen Oxidation to Nitrite. Science **1987**, *235*, 473.
18. Fidler, I.J.; Ichinose, Y. Mechanisms of Macrophage-Mediated Tumor Cell Lysis: Role for the Monokines Tumor Necrosis Factor and Interleukin-1. Immun. Cancer **1989**, *1*, 69.

19. Chen, L.; Suzuki, Y.; Wheelock, E.F. Interferon-Gamma Synergizes with Tumor Necrosis Factor and with Interleukin 1 and Requires the Presence of both Monokines to Induce Antitumor Cytotoxic Activity in Macrophages. J. Immunol. **1987**, *139*, 4096.
20. Boehm, U.; Klamp, T.; Groot, M.; Howard, J.C. Cellular Responses to Interferon-gamma. Ann. Rev. Immunol. **1997**, *15*, 749.
21. Landmann, R.; Knopf, H.P.; Link, S.; Sansano, S.; Schumann, R.; Zimmerli, W. Human Monocyte CD14 is Upregulated by Lipopolysaccharide. Infect. Immun. **1996**, *64*, 1762.
22. Landmann, R.; Ludwig, C.; Obrist, R.; Obrecht, J.P. Effect of Cytokines and Lipopolysaccharide on CD14 Antigen Expression in Human Monocytes and Macrophages. J. Cell Biochem. **1991**, *47*, 317.
23. Lichtman, S.N.; Wang, J.; Lemasters, J.J. LPS Receptor CD14 Participates in Release of TNF-alpha in RAW 264.7 and Peritoneal Cells But Not in Kupffer Cell. Am. J. Physiol. **1998**, *275*, G39.
24. Yang, R.B.; Mark, M.R.; Gray, A.; Huang, A.; Xie, M.H.; Zhang, M.; Goddard, A.; Wood, W.I.; Gurney, A.L.; Godowski, P.J. Toll-like Receptor-2 Mediates Lipopolysaccharide-induced Cellular Signaling. Nature **1998**, *395*, 284.
25. Chow, J.C.; Young, D.W.; Golenbock, D.T.; Christ, W.J.; Gusovsky, F. Toll-like Receptor-4 Mediates Lipopolysaccharide-induced Signal Transduction. J. Biol. Chem. **1999**, *274*, 10,689.
26. Jules, A.; Hofmann, F.C.; Kafatos, C.A.; Ezekowitz, R.A.B. Phylogenetic Perspectives in Innate Immunity. Science **1989**, *284*, 1313.
27. Kopp, E.B.; Medzhitov, R. The Toll-receptor Family and Control of Innate Immunity. Curr. Opin. Immunol. **1999**, *11*, 13.
28. Elferink, J.G.R.; De Koster B.M. N-acetylcysteine Causes a Transient Stimulation of Neutrophil Migration. Immunopharmacology **1998**, *38*, 229.