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# Enhanced Immune Cell Functions and Cytokine Production after *in vitro* Stimulation with Arabinoxylans Fraction from Rice Bran

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**Abstract** Arabinoxylan, a complex polysaccharide in cereal cell walls, has recently received research attention as a biological response modifier. The immunomodulating effect of arabinoxylans from rice bran (AXrb) was studied using a combined process of extrusion and commercial hemicellulase treatment in order to elucidate the augmentation mechanism of cell-mediated immunity *in vitro*. The cytotoxicity of mouse spleen lymphocytes against YAC-1 tumor cells was significantly enhanced by treatment with AXrb at 10-100 μg/mL. In an attempt to investigate the mechanism by which AXrb enhance NK cytotoxicity, we examined the effect of AXrb on cytokine production by spleen lymphocytes. Culture supernatants of the cells incubated with AXrb were collected and analyzed for IL-2 and IFN-γ synthesis by ELISA. IL-2 and IFN-γ production were increased significantly. These results suggest that AXrb may induce Th1 immune responses. Macrophages play an important role in host defenses against tumors by killing them and producing secretory products, which protect against bacterial, viral infection and malignant cell growth. AXrb were examined for their ability to induce secretory and cellular responses in murine peritoneal macrophages. When macrophages were treated with various concentrations (10-100 μg/mL) of AXrb, AXrb induced tumoricidal activity, as well as increasing phagocytosis and the production of NO, $H_2O_2$, TNF-α, IL-1β, and IL-6. These results indicate that reactive oxygen species, reactive nitrogen species, and inflammatory cytokines are likely to be the major mediators of tumoricidal activity in AXrb-treated macrophages. Therefore, AXrb may be useful in cancer immunotherapy and it is anticipated that AXrb obtained using extrusion and subsequent enzyme treatment can be used as an ingredient in nutraceuticals and cereal-based functional food.

**Keywords:** rice bran arabinoxylans, immunomodulator, cytokine

## Introduction

There is an increasing body of evidence implicating the natural killer (NK) phenomenon as a discrete subpopulation of lymphocytes capable of mediating lysis of a variety of tumor target cells regardless of major histocompatibility components (1). The various immunological functions of NK cells make them prime candidates as therapeutic agents. Interleukin-2 (IL-2) has been shown to boost NK activity in peripheral blood, both *in vitro* and *in vivo*. These activated NK cells have broader antitumor cytolytic capabilities, including lysis of fresh, uncultured, human tumor cells as well as a wide variety of tumor cell lines (2). Activated NK cells are defined as lymphokine activated killer (LAK) cells. Together, IL-2 and LAK cells have been used as adoptive immunotherapy against cancer (3). Macrophages are capable of killing tumor cells by a number of mechanisms after being activated with various agents. The tumor cell killing activity by macrophage occurs through a variety of effector pathways including the release of cytolytic protease (4). The production of reactive oxygen species (ROS), reactive nitrogen species (RNS), and cytolytic cytokines were also involved in that pathway.

Many attempts have been made to strengthen the power of the immune system using different biological response modifiers (BRMs). These are substances originating from bacteria and fungi which possess immuno-augmentative properties (5). However, there are two problems associated with these BRMs: firstly toxicity and secondly the development of hyporesponsiveness in which a single administration of the BRM can significantly enhance NK cell activity. However, it has been reported that repeated administration of the same BRM depresses NK cell activity (6). Recently MGN-3, a modified arabinoxylan from rice bran, has been reported to be a potent BRM that is able to enhance NK cell activity in cancer patients (7). It is interesting to note that MGN-3 has advantages over other BRMs. It is nontoxic and has not shown hyporesponsiveness in the four years that patients have been followed (7). Arabinoxylan is an ingredient that occurs in significant quantities in true grasses such as rice shoots, wheat, and corn. It is the principal ingredient of hemicellulose, which creates cell walls. The degenerated arabinoxylan produced by using carbohydrase complex activates the NK cell which has been reported to increase an individual's immune power through oral administration, thereby controlling the growth of cancerous cells (8). The usefulness of MGN-3 administration in treatment for AIDS (HIV) patients is also recognized (9).

In this study, the immunomodulating effect of arabinoxylans from rice bran (AXrb) was studied using a combined process of extrusion and commercial hemicellulase treatment in order to elucidate the mechanism of augmentation of cell-mediated immunity *in vitro*.

## Materials and Methods

**Preparation of rice arabinoxylan** Arabinoxylan was prepared by extrusion of rice bran followed by hemi-

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cellulase treatment of insoluble rice bran cell wall (10). Rice bran (Sinyang Co., Korea) was extruded at a screw speed of 140 rpm, a moisture content of 30%, and a feed rate of 35 kg/hr using a twin screw extruder (DNDL-40, Bühler Brothers Co., Switzerland) with an L/D ratio of 20. The detailed specifications of extrusion can be found in Hwang et al. (11). Extruded rice bran was defatted by extraction with a chloroform-methanol (2:1, v/v) solution followed by refluxing for 2 hr at 50°C. The pellets were resuspended after filtration of the suspension. This procedure was repeated twice. After addition of 1 mL of Termamyl®120 L (Novo Nordisk A/S, Denmark) per 100 g of defatted rice bran, the suspension was incubated for 2 hr at 95°C with continuous stirring. After cooling to 50°C, the rice bran suspension was allowed to react with 0.5 mL of Neutrase®500 mL (Novo Nordisk A/S, Denmark) for 5 hr with continuous stirring. The suspension was then boiled for 15 min to inactivate the enzymes. The residual solids were collected by filtration, washed with distilled water, and freeze-dried rice bran was dispersed in deionized water (1 g/20 mL) and incubated with 0.01 mL of hemicellulase Fiberase R (NewTree Co., Korea) at 50°C for 24 hr. This solution was heated to 100°C for 15 min to inactivate enzymes, cooled, then centrifuged (10,000 × g, 15 min) to remove residual solids. Low molecular weight compounds in enzyme hydrolysates were removed using a thin channel ultrafiltration system (Amicon TCF-10; Amicon Co., USA) with a MWCO 1,000 membrane. The obtained fraction was freeze-dried and thereafter referred to as AXrb (Ed- already defined above). All reagents were purchased from Sigma (St. Louis, MO, USA), unless otherwise stated. All tissue culture reagents, the thioglycollate broth, and polysaccharides were assayed for endotoxin contamination using the *Limulus amoebocyte* lysate test (E-toxate, Sigma, USA) and found to be less than 10 pg/mL.

**Animals and cell culture** Specific, pathogen-free, male BALB/c mice were purchased from Samtaco Co. (Korea). The mice, which were 4-5 weeks of age, were acclimated for at least 1 week. Mice at 5-6 weeks of age were used in this study. The animal quarters were strictly maintained at 22±2°C and 50% relative humidity and followed a twelve-hour light/dark cycle. YAC-1 and B16 tumor cell lines (Korean Cell Line Bank) were cultured in complement medium (CM), consisting of RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY), 100 IU/mL penicillin, and 100 μg/mL streptomycin (RPMI-FBS) and maintained at 37°C in a humidified incubator with 5% $CO_2$.

**Isolation of splenic lymphocytes and peritoneal macrophages** All procedures were conducted under aseptic conditions. Mice were sacrificed under ether anesthesia and the single cell suspension was prepared by pressing the spleen between two slide glasses. The cell suspensions were passed through a 200-gause stainless steel sieve and then let to stand to remove the tissue fragments. The cells were freed of red blood cells by treatment with lysis buffer (0.15 M $NH_4Cl$, 0.01 M $KHCO_3$, 0.1 mM $Na_2EDTA$, pH 7.4). To remove adherent cells such as macrophages, total spleen cells were incubated for 1 hr in petri dishes. The suspended cell populations were collected and washed twice in cold RPMI 1640 and centrifuged on Lympholyte®-Rat (Cedarlane, Canada) at 1000×g for 20 min. The cells at the interphase were collected, washed three times in RPMI 1640, and resuspended in CM. The viability of splenic lymphocytes determined by trypan blue exclusion test was >98%.

Peritoneal macrophages were obtained from mice that had been injected intraperitoneally 3 days previously with 2 mL of thioglycolate broth (Difco, USA). Following lavage of the peritoneal cavity with 6 mL of RPMI 1640, the cells were washed twice and resuspended in CM (12). Macrophages were seeded at densities of $2 \times 10^5$ cells/well on a 96-well plate (Falcon Plastics, Oxnard, CA) and allowed to adhere for 2 hr at 37°C in a humidified incubator with 5% $CO_2$. After 2 hr incubation, nonadherent cells were removed by washing with medium three times. The viability of the detached cells was assessed by trypan blue exclusion test and the proportion of macrophages was determined by a fluorescence microscope examination of acridine orange stained cells. More than 98% of the adherent cell populations were macrophages according to morphology and phagocytic criteria (13). Cells were adjusted at $2 \times 10^5$ per well, exposed to AXrb (10-100 mg/mL), and incubated for 48 hr in a humidified incubator containing 5% $CO_2$ at 37°C. Concanavalin A (Con A) and lipopolysaccharide (LPS), mitogens for T cell and macrophage, respectively, were used as positive control.

**Mitogenic activity assay** Cell viability was determined using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) reagent (14). Mitogenic activity (viability) was calculated as the percentage of control. This assay method has been reported to yield similar results to those of the traditional $^3$H-thymidine incorporation method (15). After culture on a 96-well plate with samples for 48 hr, 50 μL of 5 mg/mL MTT was added and incubated for 2 hr at 37°C. After centrifuging the plate at 200 × g for 5 min, the supernatant was aspirated off. The remaining formazan crystals formed in the viable cells were solubilized by addition of 150 μL of dimethylsulfoxide (DMSO) followed by shaking the plates on a plate rotator for 30 min at room temperature. The absorbance at 540 nm was read with a microplate reader.

**Immune cell-mediated cytotoxicity** The immune cell-mediated cytotoxicity was determined by modification of the technique described previously (16). Briefly, splenic lymphocytes and peritoneal macrophages ($1\times10^5$ cells/well) were cultured with samples for 24 hr and then co-incubated with YAC-1 and B16 melanoma cells ($1\times10^4$ cells/well; an initial effector: target cell ratio of 10:1), respectively, for 24 hr. After incubation, 50 μL of 5 mg/mL MTT was added and incubated for 2 hr at 37°C. The plate was centrifuged at 200 × g for 5 min and the supernatant was aspirated off. Formazan produced was dissolved in DMSO and the optical density of each well at 540 nm was determined using a microplate reader. Cytolytic activity was expressed as the percentage of tumor cytotoxicity where % cytotoxicity = {1 - [OD of (effector cells + target cells) - OD of effector cells] ÷ OD of target cells} × 100.

**Determination of phagocytosis** The phagocytic activity was measured using the assay system described previously (17). Cells were incubated with 250 μg/mL of zymosan and 600 μg/mL of nitroblue tetrazolium (NBT). After 1 hr incubation, plates were centrifuged at 4°C to stop the ingestion of zymosan and the 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 microplate reader (Molecular Devices Co., USA). The formazan did not need to be solubilized before the absorbance was measured.

**Assay of macrophage lysosomal phosphatase and myeloperoxidase activity** Lysosomal phosphatase activity in peritoneal macrophages was determined using *p*-nitrophenyl phosphate as substrate and using a modified version of the methods reported in Padmaja et al. (18). The cell layer was washed with RPMI1640 and solubilized with 0.1% Triton X-100. The microtiter plate was then incubated with 200 μL of *p*-nitrophenyl phosphate liquid substrate (Sigma, St, Louis, USA) in a humidified incubator containing 5% $CO_2$ at 37°C for 30 min. The reaction was stopped by the addition of 50 μL of 0.2 M borate buffer (pH 9.8) and the absorbance was measured at 405 nm using ELISA reader.

For the measurement of myeloperoxidase activity, peritoneal macrophages were washed three times with fresh medium, and the mixture (100 μL) of *o*-phenylene diamine (0.4 mg/mL) and 0.002% $H_2O_2$ in phosphate-citrate buffer (pH 5) was added. The reaction was stopped after 10 min using 0.1 N $H_2SO_4$ and the optical density at 492 nm was measured (19).

**Determination of nitrite and hydrogen peroxide ($H_2O_2$) produced by macrophages** Nitrite production, an indicator of nitric oxide (NO) synthesis, was measured in the culture supernatant of macrophages, as previously described by Kleinerman *et al.* (20). Briefly, after mixing 100 mL of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamide in 5% phosphoric acid) with 100 mL culture supernatant, the optical density at 540 nm was measured by using a microplate reader (Molecular Devices Co., USA). The $H_2O_2$ assay was based on HRP (horseradish peroxidase)-dependent oxidation of phenol red by $H_2O_2$ leading to the formation of a colored compound that at an alkaline pH exhibited increased absorbance at 600 nm (21). Briefly, cells were incubated in Hank's buffered salt solution (HBSS) with HRP 20 U/mL and phenol red 0.56 mM under an atmosphere of 5% $CO_2$ for 1 hr. After this period the reaction was stopped by adding NaOH (10 μL/well 1N NaOH) and the amounts of $H_2O_2$ formed were measured at 600 nm in the microplate reader.

**Cytokine determination by ELISA** Cell supernatants were assayed for IL-2, IFN-γ, TNF-α, IL-1β, and IL-6 using commercially available, enzyme-linked immunosorbent assay (ELISA) kits (R&D System, USA) according to the manufacturer's instructions. For all assays, 50 μL of supernatants was added to 50 μL of assay buffer. The plate was then incubated for 2 hr at 37°C after which the wells were aspirated and rinsed 4 times. After this, 100 μL of biotinylated anti-cytokine was added to each well for 1 hr at room temperature. The wells were then aspirated and rinsed 4 times after which 100 μL of streptavidin-HRP was added and the plate incubated for 30 min at room temperature until addition of the stop solution. All samples and standards were run in duplicate. The plates were read at 450 nm and the unknown samples were compared against the standard curve.

**Statistical analysis** The results are expressed as mean ± SEM. Statistical analyses were performed using ANOVA followed by Dunnett's *t*-test ($p<0.05$), with SAS statistical software.

## Results and Discussion

**Viability and NK cytotoxicity of spleen lymphocytes** When AXrb were evaluated for cell viability by MTT assay, AXrb-treated splenocytes had no cytotoxicity at concentrations ranging from 10 to 100 μg/mL (Fig. 1a). We then investigated the *in vitro* effect of AXrb on the cytotoxicity of spleen lymphocytes against YAC-1 cells which are known to be sensitive to the killing of NK and LAK cells. Culture of spleen lymphocytes with AXrb (10-100 μg/mL) enhanced NK cytotoxicity in a concentration-

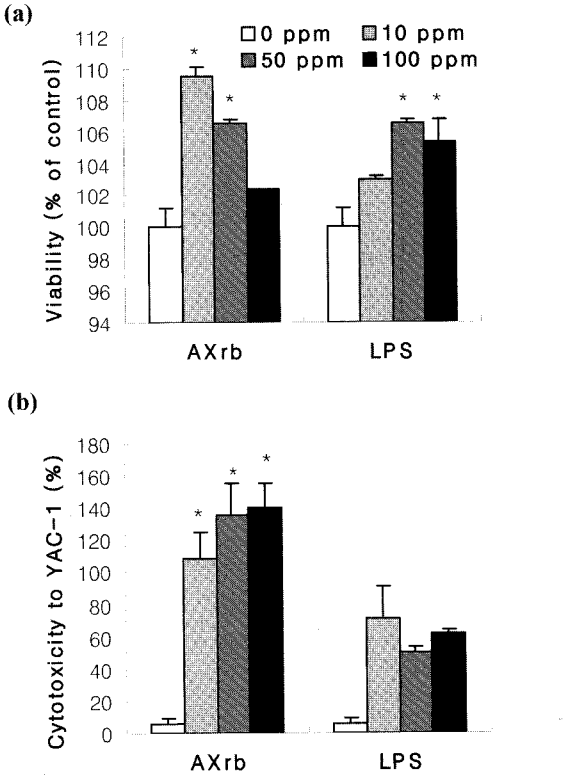


**Fig. 1. Viability and tumoricidal activity of spleen lymphocytes treated with rice bran arabinoxylans (AXrb).** Splenic lymphocytes were stimulated with AXrb for 48 hr. Viability (mitogenic activity) is expressed as the percentage of the test absorbance relative to the control absorbance at 540 nm (a). Cytotoxicity of spleen lymphocytes against YAC-1 tumor cells was determined as described in materials and methods at an initial effector/target ratio of 10:1 (b). Concanavalin A (Con A) was used as a positive control. The results are expressed as means±SEM (n=6). Significant differences from control; $^*p<0.05$.

dependent manner (Fig. 1b). AXrb at a concentration of 10 μg/mL increased NK activity by 108%. NK activity was further enhanced by 141% when the AXrb concentration was increased to 100 μg/mL. The results showed that AXrb are a potent BRM as manifested by the significant induction of NK cytotoxicity upon AXrb treatment - the augmentative action was detected against sensitive YAC-1 targets. It is well established that the NK-tumor cell interaction proceeds through several discrete stages (22) including effector: target cell recognition and binding, triggering and activation of the NK cells, release of the granules from NK cells, binding to receptor sites on the tumor cell surface, and tumor target cell death. The type of AXrb-activated killer cells is not fully defined, but it may be similar to the IL-2-induced LAK cell phenomenon with respect to heterogeneity. NK cells may represent a major component of AXrb-activated cells, as seen in the increased lysis of the classical NK-sensitive-YAC-1, but the spleen lymphocytes used in this study may involve either NK cells and/or other effector cells having anticancer activity such as cytotoxic T-lymphocytes.

**Viability, tumoricidal activity and phagocytosis of peritoneal macrophages** Macrophages are known to play an important role in host defense mechanisms by protecting against microbial invaders and tumor cells (23). AXrb showed mitogenic activity in macrophages at concentrations ranging from 10 to 100 μg/mL (Fig. 2a). We examined whether AXrb affect the tumoricidal activity of murine peritoneal macrophages against B16 tumor cells which were used as targets since they are either TNF-α or NO sensitive. Macrophages were co-incubated with B16 tumor cells in the presence of AXrb (10-100 μg/mL) for 24 hr. Tumoricidal activity of AXrb-treated macrophages was increased significantly ($p<0.05$) as compared with that of the control group (Fig. 2b). This implicates that AXrb may activate macrophage and induce macrophage-mediated, tumor killing activity. It is well known that macrophages carry out their nonspecific defense function through what is known as the phagocytic process (23). In this study, the effects of AXrb on phagocytosis were studied in relation to phagocytic activity as well as the lysosomal phosphatase and myeloperoxidase activities of the peritoneal macrophages (Fig. 3). AXrb (10-100 μg/mL) were shown to increase phagocytic activity and stimulate lysosomal phosphatase and myeloperoxidase activities of macrophages significantly ($p<0.05$). The results suggest that AXrb activate the macrophages through modulation of lysosomal enzymes activities in macrophages.

**Production of NO and $H_2O_2$ in peritoneal macrophages** When macrophages are stimulated with foreign agents, a variety of chemicals and cytokines are released to induce fundamental defense systems (23). Recent reports have suggested that NO and $H_2O_2$ are cytotoxic effector molecules of macrophage-mediated tumoricidal and bactericidal actions (24). Moreover, these cytolytic macrophages could be able to secrete various cytokines such as tumor necrosis factor-α (TNF-α), IL-1β, and IL-6 (25). Secretion of these mediators was found to require a triggering signal, thus providing some evidence for the role of the priming and triggering signals to induce the complete cytolysis function. To determine the effect of AXrb on production of ROS/RNS from macrophages, murine peritoneal macrophages were treated with various concentrations (10-100 μg/mL) of AXrb for 48 hr. Culture supernatant was tested as previously described method. Both NO and $H_2O_2$ productions were significantly (both, $p<0.05$) increased by the treatment with AXrb in comparison with that of the control group (Fig. 4a and 4b). These data imply that NO and $H_2O_2$ are important in the tumoricidal activities of AXrb-treated macrophages.

**IL-2 and IFN-γ production of spleen lymphocytes** Fig. 5 summarizes the results the AXrb effect on the production of IL-2 and IFN-γ. When spleen lymphocytes were incubated with AXrb (10-100 mg/mL) for 48 hr, IL-2 production was significantly increased in AXrb-treated splenocytes, while AXrb treatment at 50 μg/mL significantly increased IFN-γ production. The mechanism by which AXrb boost NK activity appears to result from its ability to induce interferon (IFN) production. Most agents that can activate NK cells appear to be acting via their ability to induce IFN production (26). Our work has shown that AXrb induce lymphocyte IFN-γ production in culture.

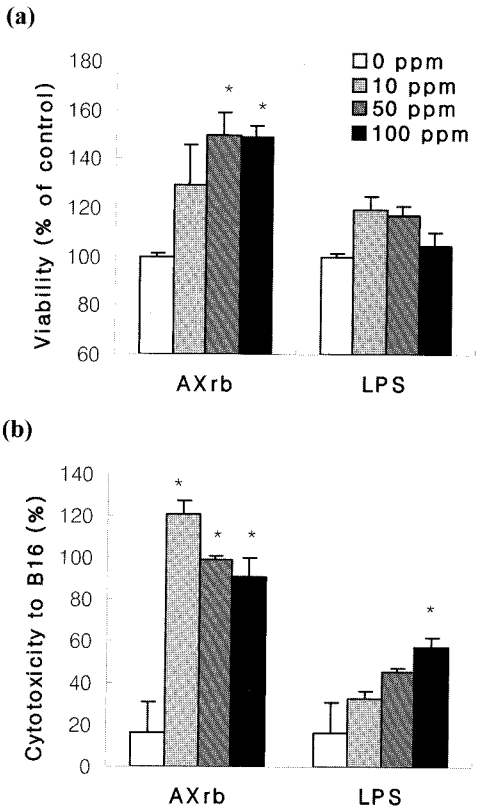


**Fig. 2. Viability and tumoricidal activity of peritoneal macrophages treated with rice bran arabinoxylans (AXrb).** Peritoneal macrophages were stimulated with AXrb for 48 hr. Viability (mitogenic activity) is expressed as the percentage of the test absorbance relative to the control absorbance at 540 nm (a). Cytotoxicity of peritoneal macrophages against B16 tumor cells was determined as described in materials and methods at an initial effector/target ratio of 10:1 (b). Lipopolysaccharide (LPS) was used as a positive control. The results are expressed as means ± SEM (n=6). Significant differences from control; $^*p<0.05$.

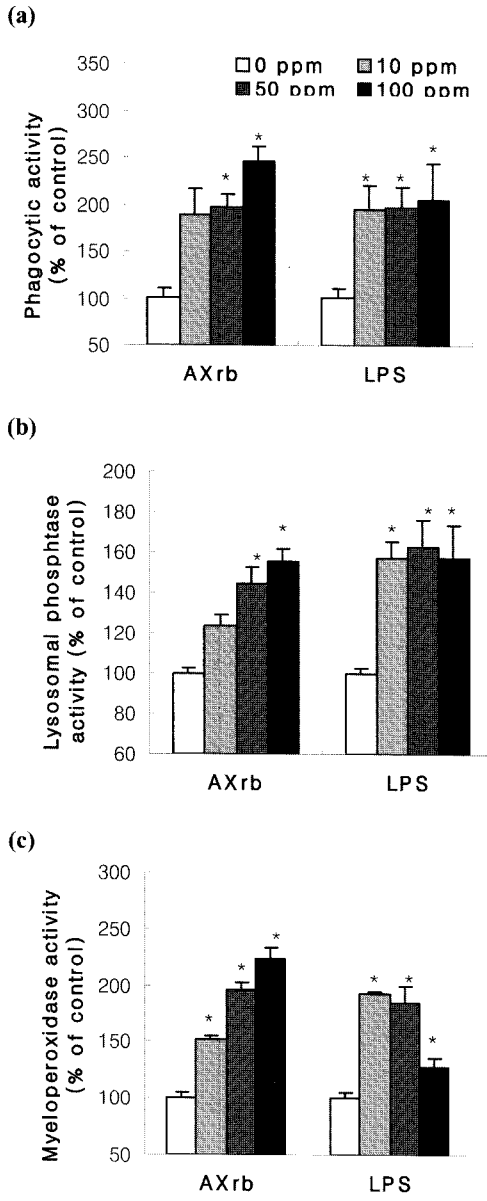


**Fig. 3. Effect of rice bran arabinoxylans (AXrb) on the phagocytic, phosphatase and myeloperoxidase activities of peritoneal macrophages.** Peritoneal macrophages were incubated with AXrb for 48 hr. Results are expressed as means ± SEM (n=6). The control values of phagocytic, phosphatase, and myeloperoxidase activities were 0.06 OD/1×10$^5$ cells, 0.75 OD/1×10$^5$ cells, and 0.10 OD/1×10$^5$ cells, respectively. Significant differences from control; $^*p<0.05$.

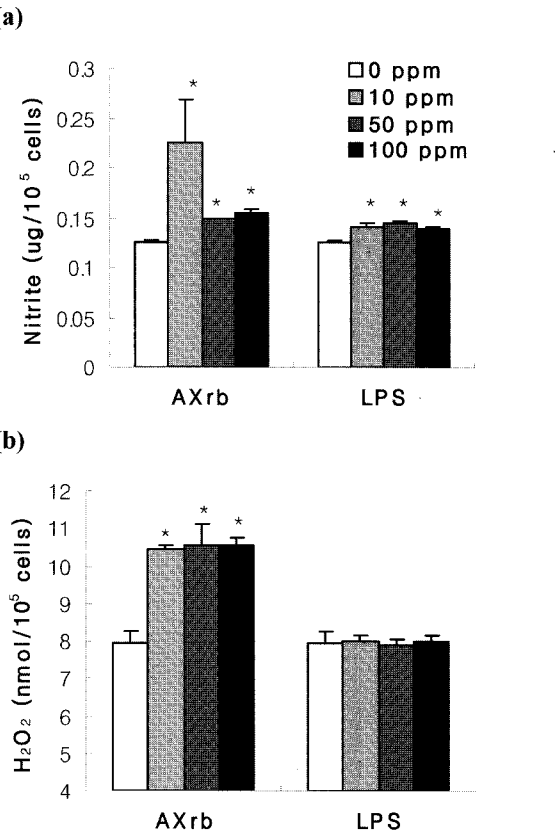


**Fig. 4. Effect of rice bran arabinoxylans (AXrb) on the nitrite and $H_2O_2$ production of peritoneal macrophages.** Peritoneal macrophages were incubated with AXrb for 48 hr. The results are expressed as means ± SEM (n=6). Significant differences from control; $^*p<0.05$.

This suggests that AXrb enhance IFN production, which in turn augments NK activity. Suppression of NK activity in cancer patients was related to defective lymphokine production (27). Our findings suggest that the augmentation of NK function could possibly result in the release of IL-2 and IFN-γ with a consequent enhancing of lymphocyte proliferation. In addition, the AXrb-induced mitogenic activity may continuously induce the secretion of IL-2. IL-2 is an important cytokine that regulates proliferation and differentiation of lymphocytes. During HIV-infection, IL-2 production is significantly reduced (28). The admini-stration of IL-2 in individuals infected with HIV-1 is expected to improve immune responses by stimulating immunocompetent cells to proliferate (29). It has been noted that treatment with IL-2 significantly increased the number of CD4+ cells in HIV-infected individuals (30). Enhancement of IL-2 in the supernatants from the cultures of spleen lymphocytes in the presence of AXrb implied the possibility for AXrb to induce IL-2 secretion from immune-deficient patients. Arabinoxylan has been reported to have an enhancement effect on NK cell activity in human peripheral blood lymphocytes (8). Moreover, it is known that increased NK cell activity is induced by IFN-γ and IL-2 (31). Hence, our results support that treat-ment of AXrb enhances NK cell activity in lymphocytes. The mechanism by which AXrb augment the cytotoxic activity of lymphocytes remains unclear, but it could be due to the stimulation of cytokine production (TNF-α, IFN-γ, IL-1β) since activated cytotoxic T cells develop the capacity to transcribe and secrete cytokines and other proteins to kill the tumor cells (32). LAK cells are important effector cells for cell-mediated, anti-tumor activity. In the present report, AXrb were found to significantly increase IL-2 production and augment the cytotoxic activity of spleen lymphocytes *in vitro*. Probably, AXrb mimic the T cell stimulatory effect of IL-2 on the splenic LAK cells of NK cell origin (33). T helper cells are divided into Th1 cells and Th2 cells from the profile of cytokines secretion (34). It is well known that Th1 cells able to produce IL-2 and IFN-g, whereas Th2 cells can

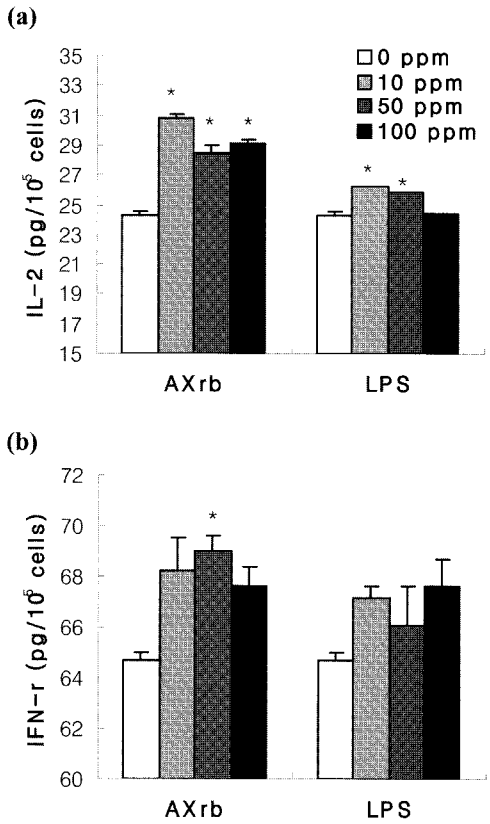


**Fig. 5. Effect of rice bran arabinoxylans (AXrb) on the cytokines production of spleen lymphocytes.** Splenic lymphocytes were incubated with AXrb for 48 hr. Production of cytokines in culture supernatants was measured by ELISA system. The results are expressed as means ± SEM (n=6). Significant differences from control; $^{*}p<0.05$.

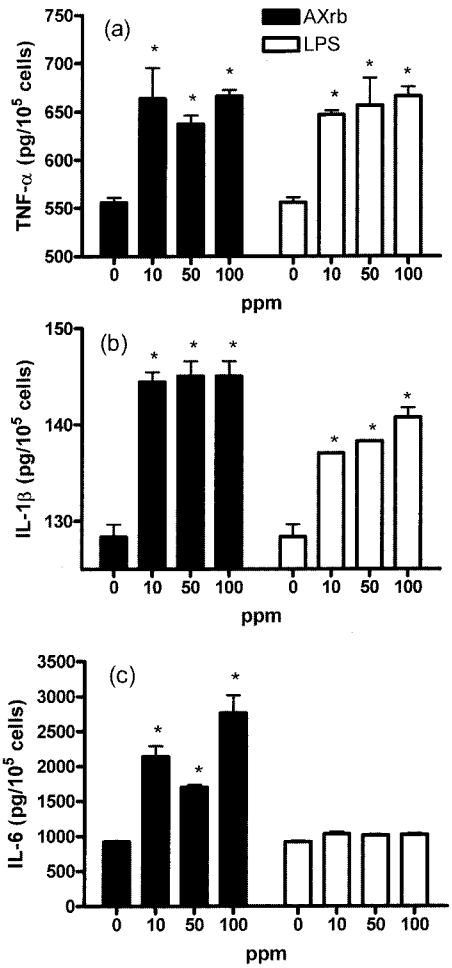


**Fig. 6. Effect of rice bran arabinoxylans (AXrb) on the cytokines production of peritoneal macrophages.** Peritoneal macrophages were incubated with AXrb for 48 hr. Production of cytokines in culture supernatants was measured by ELISA system. The results are expressed as means ± SEM (n=6). Significant differences from control; $^{*}p<0.05$.

produce IL-4 and IL-10. Th1 cells upregulate mainly cell-mediated immunity and downregulate humoral immunity, whereas Th2 cells act oppositely (35). In the current study, we observed that AXrb enhanced the production of IL-2 and IFN-γ by Th1 cells. Therefore, it might be speculated that AXrb treatment augments cell-mediated immunity and that AXrb-stimulated cytotoxic T cells may play an important role in the cell-mediated anti-tumor immunity of the host.

**Production of cytokines in peritoneal macrophages** To determine the effect of AXrb on the production of TNF-α, IL-1β, and IL-6, macrophages were treated with various concentrations (10-100 μg/mL) of AXrb for 48 hr. Culture supernatants were assayed for cytokines by ELISA. The production of TNF-α, IL-1β, and IL-6 was significantly increased by the treatment with AXrb ($p<0.05$) as compared to that of control (Fig. 6). These results suggest that TNF-α, IL-1β, and IL-6 are important cytokines in the tumoricidal activities of AXrb-treated macrophages. TNF-α produced by activated macrophages and other cell types, including T and B cells, NK cells and LAK cells, plays a critical role in normal host resistance to infection and to the growth of malignant tumors, serving as an immunostimulant and as a mediator of the inflammatory response (36). IL-1β is one of the representative cytokines secreted by macrophages to play a key role in the cytokines network, e.g., T-cell activation (23). A decline of this cytokine weakens the resistance to infectious diseases (37). In this study, AXrb have activated murine peritoneal macrophages, thereby increasing the production of various molecules such as NO, $H_2O_2$, TNF-α, IL-1β, and IL-6. These results suggest that the beneficial, immunostimulatory activity of AXrb may be mediated through upregulation of secretory molecules in macrophages, and the AXrb may prevent the organism from foreign agents through enhancement of secretion of these inflammatory cytokines by peritoneal macrophages.

Polysaccharides such as pectic silenans have been shown to increase the uptake capacity of rat peritoneal macrophages and have been suggested to enhance expression of macrophage Fc receptor like pectic polysaccharides of *Bupleurum falcatum* (38) and *Panax ginseng* (39). It can be supposed that LPS receptor CD14 participates in macrophage activation by plant polysaccharide. CD14 recognizes a variety of carbohydrate-containing ligands, including uronic acid polymers (40). It is possible that membrane-associated events may be involved in the AXrb-mediated immunostimulating

process.

Our results demonstrated that AXrb stimulated spleen lymphocytes and increased the production of IL-2 and IFN-γ, which could augment the NK cytotoxicity. In addition, AXrb treatment altered the cellular functions of macrophages to kill tumor cells and to produce various molecules such as NO, $H_2O_2$, TNF-α, IL-1β and IL-6. These results suggest that AXrb may induce Th1 immune responses and that ROS, RNS and inflammatory cytokines are likely to be the major mediators of tumoricidal activity in AXrb-treated macrophages. Therefore, AXrb may be useful in cancer immunotherapy and it is anticipated that AXrb obtained using extrusion and subsequent enzyme treatment can be used as an ingredient in nutraceuticals and cereal-based functional food. Further studies are needed to clarify the molecular mechanisms of the arabinoxylan-enhanced, immune function.

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