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Antitumor and Immunomodulating Activities of the Polysaccharide Fractions from *Artemisia selengensis* and *Artemisia iwayomogi*

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Effects of the polysaccharide fractions purified from *Artemisia selengensis* and *Artemisia iwayomogi* on the immune system was studied. The polysaccharide fractions, respectively called ASP1 and AIP1, may interact with macrophages and lymphocytes in spleen, increasing the population of those cell types *in vivo* and *in vitro*. Both ASP1 and AIP1 fractions also suppress transplanted tumor cell growth and augment antibody production. This study suggests that ASP1 and AIP1 fractions may have immunomodulating and antitumor activities.

Key words: *Artemisia selengensis, Artemisia iwayomogi,* Macrophage, Lymphocyte, Immunomodulating, Antitumor

INTRODUCTION

It is well known that large number of the plant polysacchrides have various biological activities. Polysaccharides from *Acanthopanax obovatus* root showed immunomodulating and antitumor activities (Wang *et al.*, 1993). Polysaccharides from *Sanguisorba officinalia* (Kim *et al.*, 1993) and *Anemarrhena asphodeloides* (Takahashi *et al.*, 1985) have been reported to show anticoagulant activity and hypoglycemic activity, respectively. Other polysaccharides are also known to possess antitumor, immunomodulating, antiinflammatory, hypoglycemic and antiviral activities (reviewed by Srivastava and Kulshreshtha, 1989).

Artemisia selengensis and *Artemisia iwayomogi*, the members of the Family Compositae, are perennial herbs easily found around Korea. They are respectively called "*yugino or mulsuk*" and "*haninjin or dowijigi*" in Korea and are traditionally used for treatment of the various liver diseases (Lee *et al.*, 1993). Polysaccharides from *Artemisia princeps* showed anticomplementary activity (Yamada *et al.*, 1984), which suggest prospective immunomodulating activity of the plants in this Genus. We previously showed that the polysaccharides purified from the leaves of these plants extended the survival of the murine spleen cells *in vitro* (Lee *et al.*, 1993). In that study, the active fractions of the polysaccharides, respectively called ASP1 and AIP1, were shown to have similar size of molecular weight 2,500 daltons, and to be composed of mainly glucose and uronic acids.

Macrophages and monocytes are the cells of the mononuclear phagocyte system, which constitutes one of the major cell population of the immune system. Monocyte is the cell in the blood, whereas same type of cells in connective tissues is called macrophage. Some of the macrophages are also called various other names such as microglia, reticular cells and Kupffer cells depending on the type of tissue they reside. Macrophages play major role in specific acquired immunity as well as in natural immunity. They function in natural immunity by phagocytosis of foreign invaders such as microbes. Macrophages also function in specific acquired immunity by displaying antigens on their surface to initiate helper T cell activation. The helper T cells then stimulate B cells to be the plasma cells, which produce antibodies. Macrophages produce various helper cytokines to stimulate other immune cells including helper T cells, and they are also part of the major effector cells in cellular immunity (reviewed by Abbas *et al.*, 1993).

According to the immune surveillance theory, the immune system can recognize and destroy tumor cells expressing tumor specific antigen (Burnet, 1970). Al-

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though antibodies, natural killer cells and cytotoxic T cells are well known as the major effector mechanisms of the antitumor immunity, macrophages are also potentially important cellular mediators of anti-tumor immunity. The activated macrophages can preferentially lyse tumor cells and also secrete the cytokine tumor necrosis factor (TNF) which can kill tumor cells. Macrophages also show augmented phagocytosis against target cells, including tumor cells, coated with antibodies (Abbas *et al.*, 1993). Here, we describe that polysaccharides ASP1 and AIP1 may activate macrophages and lymphocytes in the mouse spleen and that they may possess antitumor and immunopotentiating activities.

MATERIALS AND METHODS

Mice and Cell Lines

Eight-week-old male ICR mice were purchased from the Korea Experimental Animal Center, Seoul, Korea. Sarcoma 180 cell line was obtained from Korean Cell Line Bank, Seoul, Korea.

Chemicals

Most of the reagents used for cell culture, tumor cell inoculation and antigen injection were purchased from the Sigma Chemical Co., St. Louis, U.S.A. Alkaline phosphatase conjugated goat anti-mouse IgG and IgM were also obtained from Sigma Chemical Co.

Purification of the Polysaccharides Fractions

Crude Polysaccharide preparations from the leaves of *Artemisia selengensis* and *Artemisia iwayomogi* were purified further using Sephadex G-150 column chromatography, and the active fractions were pooled and ethanol precipitated as described previously (Lee *et al.*, 1993). The polysaccharide precipitations were then dissolved in phosphate buffered saline (PBS) and were used as ASP1 and AIP1 fractions, respectively.

Effects of ASP1 and AIP1 Fractions on the Spleen Cells *in vitro*

Spleen cells were prepared from the 8 weeks old male ICR mice and cultured in the medium supplemented with ASP1 or AIP1 fraction as described previously (Lee *et al.*, 1993). Same volume of PBS was added to the control cultures. After cultivation of the spleen cells for a month, the cells were examined morphologically by Wright staining method to determine the types of cells in the cultures (Strober, 1992).

Effects of ASP1 and AIP1 Fractions on the Spleen Cells *in vitro*

Table I. Effects of the ASP1 and AIP1 fractions on the cellular constitution of the mouse spleen cell culture. Spleen cells were cultured with ASP1 or AIP1 fraction for a month and cell types of the resulting culture were morphologically determined. The lows indicated by "AIP" and "ASP" represent the cultures supplemented with AIP1 and ASP1 fractions, respectively. The low indicated by "PBS" represents the control culture supplemented with PBS

	small lymphocytes	lymphoblasts	monocytes
AIP	60%	32%	8%
ASP	23%	51%	26%
PBS	90%	6%	4%

Prior to injection of an antigen, 1.6 mg of ASP1 or AIP1 fraction was injected intraperitoneally to groups of 8-week-old male ICR mice for 5 consecutive days. Same volume of PBS was injected to the control group of mice. After 5 days of treatment, mice were sacrificed and their spleens were removed. Cells were prepared from the spleens (Lee *et al.*, 1993), and the cellular constitutions of the spleens were examined morphologically using Wright staining method.

Evaluation of the Antitumor Activity

Groups of 8-week-old male ICR mice were also treated with ASP1 fraction, AIP1 fraction, or PBS for 5 consecutive days as described above. The mice were then followed by the intraperitoneal inoculations of 4×10^6 Sarcoma-180 cells, and survival of each mouse was scored during 60 day period thereafter.

Assay of Antibody Production

Prior to immunization, mice were pretreated with ASP1 fraction, AIP1 fraction or PBS for 5 consecutive days as described above. The mice were then immunized by intraperitoneal injection of 10 μg of keyhole lymphet hemocyanine (KLH) without an adjuvant. Ten days later, mice anti-sera were prepared, and the titres of anti-sera were determined using ELISA methods as described by Hornbeck (1992).

RESULTS AND DISCUSSION

We showed previously that the polysaccharide fractions ASP1 and AIP1 significantly extended survival of the spleen cells *in vitro* (Lee *et al.*, 1993). In order to understand the nature of that phenomenon, we decided to examine whether there was any change in the cellular constitution during spleen cell cultivation with these polysacchatide fractions. Examining the cell types in the culture, we found that the populations of lymphoblastic cells and monocytes were greatly increased by the treatment with ASP1 and AIP1 fractions

Table II. Effects of the ASP1 and AIP1 fractions on the cellular constitution of the mouse spleen cell *in vivo*. Following the treatment of mice with ASP1 or AIP1 fraction for 5 consecutive days, spleens were removed and cell types in the spleen were morphologically determined. The lows indicated by "AIP" and "ASP" represent the cellular constitutions of the spleen from the mice treated with AIP1 and ASP1 fractions, respectively. The low indicated by "PBS" represents the one of the spleen from the control mice treated with PBS.

	small lymphocytes	lymphoblasts	monocytes	others
AIP	60%	18%	17%	5%
ASP	51%	24%	25%	
PBS	88%	8%	4%	

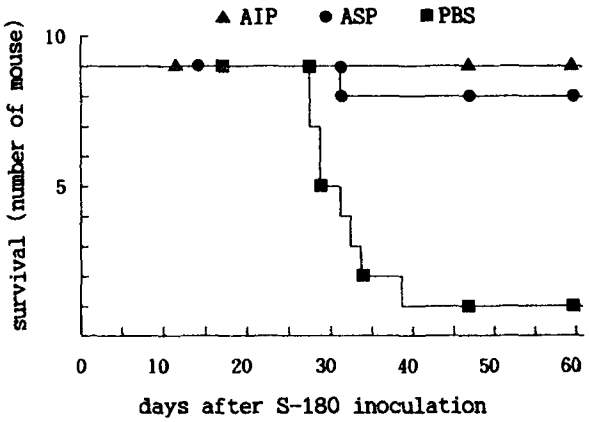

Fig. 1. Effects of the ASP1 and AIP1 fractions on survival of the mice transplanted intraperitoneally with Sarcoma-180 ascitic tumor. Following the treatment of mice with ASP1 or AIP1 fraction for 5 consecutive days, we inoculated Sarcoma-180 cells intraperitoneally into each mouse and survival of the mouse was scored. The lines marked with "circle" and "triangle" represent the mice pretreated with ASP1 and AIP1 fractions, respectively. The line marked with "rectangle" represents the control mice pretreated with PBS. The mice survived longer than 60 days looked normal even after 60 day of the tumor inoculation.

(Table I). We also treated groups of mice with these polysaccharide fractions and examined cellular constitution of the spleen to see the similar change *in vivo* too. As expected, large number of the resting small lymphocytes were activated to lymphoblasts and the population of macrophages was also expanded in the mouse spleen by the treatment, suggesting that ASP1 and AIP1 fractions may interact with lymphocytes and macrophages (Table II). The increases of the macrophage populations both *in vivo* and *in vitro* raise the following possibilities. The ASP1 and AIP1 polysaccharides might interact with macrophages and then the activated macrophages could stimulate resting lymphocytes to lymphoblasts since macrophages stimulate helper T cells by presenting antigen and producing

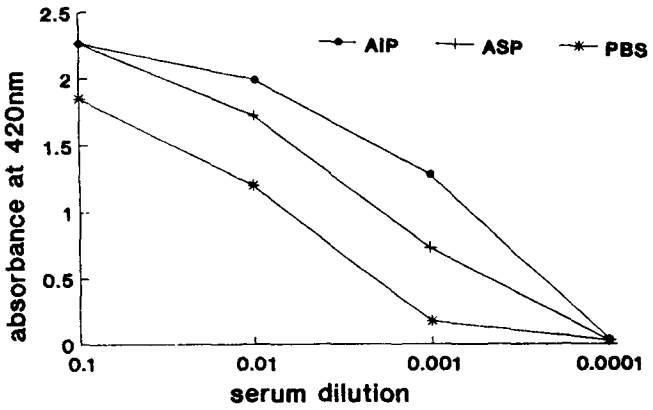

Fig. 2. Effects of the ASP1 and AIP1 fractions on primary antibody response. Following the treatment of mice with ASP 1 or AIP1 fraction for 5 consecutive days, we immunized the mice with KLH without an adjuvant, and examined the antibody titre of the resulting antisera. Each of the serum was dilutes 10 to 10000 times and the amounts of IgM class antibodies were determined by ELISA using alkaline phosphatase conjugated goat anti-mouse IgM antibody. The lines marked with "plus" and "dot" represent the mice pretreated with ASP1 and AIP1 fractions, respectively. The line marked with "asterisk" represents the control mice pretreated with PBS.

helper cytokines such as IL-1. It is also possible that these activated helper T cells then stimulate resting B cells to lymphoblasts (Abbas *et al.*, 1993). All of these interactions could end up in the increase of the population of macrophages and lymphoblasts.

It has been reported that the activated macrophages could inhibit tumor cell growth by producing tumor suppressing cytokines and selectively lysing tumor cells (Abbas *et al.*, 1993). To test whether ASP1 and AIP1 fractions suppress tumor cell growth, groups of mice were treated with these polysaccharide fractions and were followed by the intraperitoneal inoculation of the Sarcoma-180 tumor cell line. While most of untreated mice died between day 27 and day 40 of the tumor injection, all of nine mice treated with AIP1 and all but one mouse treated with ASP1 survived (Fig. 1). We also tested the possibility that the ASP1 and AIP1 fractions may possess direct cytotoxicity against Sarcoma-180 cell line. Yet we did not see any toxic effect of these polysaccharides on Sarcoma-180 cell line *in vitro*, suggesting the tumor cells were killed by the immune response modulated by the treatment with ASP1 and AIP1 fractions. These results indicate that the treatment with ASP1 and AIP1 fractions could stimulate mouse immune system and might help the mouse to clear inoculated tumor cells, since ASP1 and AIP1 were shown to interact with macrophages and lymphocytes.

We also studied the effects of ASP1 and AIP1 fractions on the antibody production against a protein antigen. Groups of mice were treated with ASP1 or AIP1

fraction and immunized by a protein antigen KLH with complete Freund's adjuvant. The mice were then immunized again with the same antigen a month later. Examining the titres of antibodies of the IgG isotype in the resulting antisera, we could not observe any differences in the antibody titres between treated and untreated groups because of good antibody production in all groups of mice. Since ASP1 and AIP1 fractions might interact with macrophages as described above and the adjuvant is also known to act on macrophage, immunization of the antigen with the adjuvant might mask stimulatory effects of the polysaccharides. Therefore, we could not see any differences in the antibody response. In addition to the adjuvant problem, we might have another problem since we had examined the secondary antibody response not the primary antibody response. In the secondary antibody response, it is well known that B cells function as major antigen presenting cell. In the primary antibody response, however, macrophages are known to function as the major antigen presenting cells (Lanzavecchia, 1990; Vitetta *et al.*, 1989). Because ASP1 and AIP1 polysaccharides may interact with macrophages to modulate immune response, we should have examined the effects of the polysaccharides on the primary antibody response where macrophages play major roles. We examined this possibility by testing antibody production in primary antibody response while antigen was injected without adjuvant. In this study, the pretreatment with ASP1 or AIP1 fraction significantly increased antibody titres than those of the untreated control mice (Fig. 2). This observation also supports that ASP1 and AIP1 fractions may act on the macrophages and may modulate their activities such as antigen presentation and T cell activation. The activated T cells then may help B cells to produce more antibodies. It is necessary, however, to purify macrophage population and to study the interaction of the macrophage population with these polysaccharides more directly. It is also of interesting to see whether ASP1 and AIP1 fractions can interact with the macrophages of the liver and thus modulate its function, since the plants has been used for treatment of various liver disease.

CONCLUSIONS

The polysaccharide fractions ASP1 and AIP1, purified from *Artemisia species*, may possess immunomodulating and antitumor activities. It is possible to speculate that the polysaccharides fractions may interact with macrophages and thus stimulate other immune cells including lymphocytes. More studies related to the macrophage activation are necessary.

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