Enhancing Effect of Antitumor Polysaccharide from *Astragalus* or *Radix hedysarum* on C3 Cleavage Production of Macrophages in Mice

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Abstract—Effects of Astragalus polysaccharide (APS) and Radix hedysari polysaccharide (RHPS) on the third component of complement (C3) cleavage production of macrophages in ICR mouse were investigated by the immunofluorescent method. By two hours after intraperitoneal injection of 500 mg/kg of APS or RHPS, there was an obvious increase in the deposition of C3 on peritoneal macrophages. When APS or RHPS was injected 5 times (1 time/day), the proportion of C3 positive macrophages was more than that of 1-time injection. At the same time, the number of peritoneal macrophages also increased. Since C3 cleavage product occurred with the activation of C3, the results suggest that the immunopotentiating action of APS and RHPS may be related to the activity of mouse complement C3.

*Astragalus membranaceus* and *Radix hedysarum polybatrys*, which belong to the same biological family but different genera, have been widely used as traditional tonic herbs in China. It has been proven that astragalus polysaccharide (APS) and *Radix hedysarum* polysaccharide (RHPS) inhibit the growth of mouse transplanted tumor, Sarcoma 180 and Ascites hepatoma, but have no effect on tumor cells in vitro. APS and RHPS not only enhanced immune function in normal mice but also prevented the immunosuppressive effects of cyclophosphamide or prednisolone (1, 2). When APS or RHPS at 500 mg/kg was intraperitoneally injected 5 times, the phagocytic activity, plaque forming cells of spleen cells and T lymphocyte transformation induced by phytohemagglutinin were potentiated both in normal and immunosuppressed mice (1, 3). Since APS and RHPS have no direct cytocidal action, the antitumor effect of APS and RHPS may be due to their immunopotentiating action. It was reported that some host-mediated antitumor agents not only increased the activity of macrophages, but also augmented complement C3 receptors on macrophages (4, 5). The activity of the complement system was shown to be associated with tumor immunity (6). However, little is known about the effect of APS or RHPS on the complement system. Therefore, in this paper we investigated the effect of APS and RHPS on C3 cleavage production of macrophages in the peritoneal cavity of mice.

APS and RHPS, which were isolated from *Astragalus membranaceus* and *Radix hedysarum polybatrys* roots (2), were dissolved in physiological saline. Eight-week old female ICR mice were intraperitoneally injected with APS or RHPS (500 mg/kg). Peritoneal macrophages were obtained from the mice 2 hr after the first injection and 2 hr after the 5th injection (1 injection/day) by washing the peritoneal cavity with Eagle's minimum essential medium (MEM). The cell suspension was centrifuged at 800 rpm for 5 min. The resulting sediment was resuspended in Eagle's MEM to 2 × 10^6 cells/ml.

A 0.2 ml portion of the cell suspension was placed on a coverslip in a petri dish and incubated at 37°C for 30 min in a 5% CO₂ incubator. The cells that did not adhere to the coverslips during the incubation were removed by rinsing with Eagle's MEM. The
adherent cells on the coverslips were fixed in 95% ethanol for 20 min, then dried, covered with anti-mouse C3 F(ab')2 and reincubated for 1 hr at 37°C. After the incubation, the cells were rinsed thoroughly with PBS (pH 7.2), stained with fluorescein isothiocyanate (FITC) labelled anti-rabbit IgG at room temperature for 1 hr, thoroughly washed again with PBS (pH 7.2), mounted in buffered glycerol (pH 9.5) and examined under a microscope (x400). About 600 adherent cells were counted on each slide, and the percent of fluorescent cells was calculated (7).

The dose of APS or RHPS used in this experiment was based on their immunopotentiating and antitumor effects in vivo (1–3). The proportion of C3 positive macrophages and the number of peritoneal macrophages were markedly increased by administration of APS or RHPS for 1 and 5 times (Table 1). When saline was intraperitoneally injected 5 times, the proportion of C3 positive macrophages were more than that of 1-time injection. The reason why the C3 positive cells were different between the two control groups may be due to physical irritating effects. However, when saline was injected 10 times, the proportion of C3 positive cells was similar to that of 5-time injection (data not shown). This means that the irritating effect of saline is limited. The effect of APS and RHPS on the proportion of C3 positive macrophages is more obvious in groups treated 5 times than in groups treated 1 time. The C3 positive macrophages increased to about 90% after administration of APS or RHPS (5 times). However, in the control group, only 11.2% showed immunofluorescence. The results indicated that effects of APS and RHPS are associated with accumulating dose.

As shown in Fig. 1, the immunofluorescence of groups treated with APS or RHPS, 500 mg/kg, 5 times is much stronger than that of the control in macrophages of ICR mice. Since APS and RHPS have no direct cytocidal action (data not shown), the antitumor effect of APS and RHPS may be due to their immunopotentiating action. It is well-known that macrophages are one of the most important cells in the immune defense. APS and RHPS not only enhanced phagocytic activity (3, 8) but also significantly increased the deposition of C3 on peritoneal macrophages as demonstrated by an indirect fluorescent antibody technique using Fc-free rabbit IgG anti-mouse C3. As peritoneal macrophages of mice are known to have mainly C3b receptors, it is considered that the C3 cleavage product on macrophages consists of C3b (9). Continued ability to bind C3 cleavage product via the C3b receptor could play an important role in the immune responses (6). It is suggested that enhancing the effect of phagocytosis and other immune responses by APS and RHPS (1–3, 8) may be related to the C3 activation in vivo. Nevertheless, although APS and RHPS are generically different, the effect of increasing C3 activation is the same as that of APS and RHPS on other immune re-

### Table 1. Effect of APS or RHPS on induction of C3-positive cells and number of mouse peritoneal macrophages

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Injection of 1 time</th>
<th>Injection of 5 times</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3.3±3.0</td>
<td>11.2±2.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.7±0.2)</td>
<td>(2.4±0.4)</td>
</tr>
<tr>
<td>Saline</td>
<td>0.4 ml</td>
<td>28.4±4.7***</td>
<td>92.0±2.1***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.5±0.5)***</td>
<td>(15.8±1.3)***</td>
</tr>
<tr>
<td>APS</td>
<td>500 mg/kg</td>
<td>23.2±6.6***</td>
<td>88.2±10.4***</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.8±0.7)***</td>
<td>(20.7±7.6)***</td>
</tr>
<tr>
<td>RHPS</td>
<td>500 mg/kg</td>
<td></td>
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</tbody>
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*In mouse peritoneal macrophages on the coverslips after treatment with APS or RHPS. ( ) Number of peritoneal macrophages (1×10⁶) per mouse. Values represent the mean±standard deviation of 5 experiments. ***P<0.001.
Fig. 1. Fluorescence photomicrograph of C3 cleavage product present on peritoneal macrophages by treatment (5 times) with saline (0.4 ml) (A), APS (500 mg/kg) (B) and RHPS (500 mg/kg) (C). x400.

responses (1, 3). Further studies on the biochemical properties of APS and RHPS are necessary to elucidate the mechanism of their antitumor and immunopotentiating action.

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References