Antitumor and immunomodulatory activity of polysaccharides from the roots of 
Actinidia eriantha

Hai-Shun Xu a, b, Yuan-Wen Wu c, Shi-Fang Xu d, Hong-Xiang Sun a, *, Feng-Yang Chen d, Li Yao b

a Key Laboratory of Animal Epidemic Etiology & Immunological Prevention of Ministry of Agriculture, College of Animal Sciences, Zhejiang University, Hangzhou 310029, China
b College of Medicine, Zhejiang Chinese Medical University, Hangzhou 310053, China
c Medical school, Jinhua College of Profession & Technology, Jinhua 321007, China
d Institute of Materia Medica, Zhejiang Academy of Medical Sciences, Hangzhou 310013, China

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ABSTRACT

Aim of the study: The roots of Actinidia eriantha Benth (Actinidiaceae) have been used for cancers in the Chinese folk medicine. The present study aimed at evaluating the antitumor potentials of the polysaccharides from the roots of Actinidia eriantha and elucidating their immunological mechanisms by determining the effects on the growth of tumor transplanted in mice and the immune response in tumor-bearing mice.

Materials and methods: The total polysaccharide AEP and four purified polysaccharides AEPA, AEPB, AEPc and AEPD were isolated and purified from the roots of Actinidia eriantha by hot water extraction, ethanol precipitation, dialysis and gel filtration. Their effects on the growth of mouse transplantable tumor, splenocyte proliferation, the activity of natural killer (NK) cells and cytotoxic T lymphocytes (CTL), production of cytokines from splenocytes, and serum antigen-specific antibody levels in tumor-bearing mice were measured.

Results: AEP and four purified polysaccharides could not only significantly inhibit the growth of mouse transplantable tumor, but also remarkably promote splenocyte proliferation, NK cell and CTL activity, IL-2 and IFN-γ production from splenocytes, and serum antigen-specific antibody levels in tumor-bearing mice.

Conclusions: The antitumor activity of AEP and four purified polysaccharides might be achieved by improving immune response, and the composition of the monosaccharides, uronic acid contents and molecular weight could affect their antitumor and immunomodulatory activity.

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1. Introduction

Malignancy is one of the most serious diseases that damage human health in the modern world and the second largest deadly disease just below heart disease. According to WHO estimates, more than 7 million people died of cancer in 2005 (Liu et al., 2006). There exists close relationship between the occurrence, growth and decline of tumor and immune states (Ehrke, 2003). The low immune function of an organism may not only result in the generation and development of a tumor, but also be one of the most important factors that prevent the tumor patients’ recovery. Immunomodulation through natural or synthetic substances may be considered an alternative for the prevention and cure of neoplastic diseases (Mitchell, 2003). The enhancement of host immune response has been recognized as a possible means of inhibiting tumor growth without harming the host (Yuan et al., 2006). Therefore, it is very important to investigate novel antitumor substances with improving immunity potential.

Most polysaccharides derived from higher plants are relatively nontoxic and do not cause significant side effects, which is a major problem associated with immunomodulatory bacterial polysaccharides and synthetic compounds (Schepetkin and Quinn, 2006). Thus, plant polysaccharides are ideal candidates for therapeutics with immunomodulatory and antitumor effects and low toxicity (Schepetkin and Quinn, 2006). Recently, lentimun, schizophyllan and krestin have been accepted as immunonutrients in several oriental countries (Wasser, 2002).

Actinidia eriantha Benth (Actinidiaceae) is a liana plant that commonly grows in temperate climate zones. Its roots have been used for gastric carcinoma, nasopharyngeal carcinoma, breast carcinoma, and hepatitis in traditional Chinese medicine (Jiangsu New Medical College, 1977). Pharmacological experiments also indicated that the water extracts of this drug possesses the antitumor and immunopotentiating activities (Lin et al., 1987).
Some triterpenes, such as ursolic acid, 2α,3α,24-trihydroxyurs-12-en-28-oic, 24-acetoxy-2α,3α-dihydroxyurs-2-en-28-oic acid (eriatic acid A), 2β,3β,23-trihydroxyurs-12-en-28-oic acid (eriatic acid B), 2α,3β-dihydroxyurs-12-en-28-oic acid, 2β,3β-dihydroxy-23-oxyurs-12-en-28-oic acid, have been isolated from Actinidia eriantha (Bai et al., 1997a,b; Huang and Chen, 1992; Huang et al., 1988). However, the antitumor constituents from the roots of Actinidia eriantha have not yet been reported. We previously compared the inhibitory effect of the water and ethanol extract from the roots of Actinidia eriantha on the growth of S180 sarcoma and H22 hepatoma transplanted in mice, and found that the inhibitory rates of the water extract on these two tumors transplanted in mice were significantly higher than those of the ethanol extract. The water extract was further subjected to D101 resin column chromatography, eluted with water and aqueous ethanol to affording four fractions. Among the eluted fractions, the water-eluted fraction showed the strongest inhibitory effect on the growth of S180 sarcoma transplanted in mice, indicating that the active principles were distributed to the water-soluble constituents. In the present study, the water extract of this drug was subjected to ethanol precipitation, dialysis, and gel column chromatography affording the total polysaccharide AEP and four purified polysaccharides, namely AEP-A, AEP-B, AEP-C, and AEP-D, as well as their antitumor and immunomodulatory activity on the immune response in tumor-bearing mice were investigated.

2. Materials and methods

2.1. Experimental animals

ICR mice (Grade II, 5 weeks old) weighing 18–22 g were purchased from Zhejiang Experimental Animal Center (Certificate No. 22–2001001, Hangzhou, China) and acclimatized for 1 week before use. Half of them were male and the others were female. Rodent laboratory chow and tap water were provided ad libitum and maintained under controlled conditions with a temperature of 24 ± 1 °C, humidity of 50 ± 10%, and a 12/12 h light/dark cycle. All the procedures were in strict accordance with the PR China legislation on the use and care of laboratory animals and with the guidelines established by Institute for Experimental Animals of Zhejiang University and were approved by the university committee for animal experiments.

2.2. Materials

Arabinose, fucose, galactose, glucose, mannose, rhamnose, xylose, glucuronic acid, triluoroacetic acid (TFA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), concanavalin A (Con A), lipopolysaccharide (LPS), and rabbit anti-mouse IgG peroxidase conjugate were purchased from Sigma Chemical Co., Saint Louis, Missouri, USA; DEAE-Sephadex A-50 and Sepharyl S-400 were from Amersham Biosciences Co., Piscataway, New Jersey, USA; RPMI-1640 medium and fetal calf serum (FCS) were from Gbco, Grand Island, NY, USA; goat anti-mouse IgG1, IgG2a, and IgG2b peroxidase conjugate were from Southern Biotech Assoc., Birmingham, AL, USA; cytokine (IL-2 and IFN-γ) detecting ELISA kits were from Wuhan Wushe Biological Technology Co. Ltd., Hubei, China. Cyclophosphamide (CTX) was provided by Jiangsu Hengrui Company, China. Inositol was purchased from Huamei Biochemistry Co., Shanghai, China. All other chemicals and solvents used were of analytical grade.

2.3. Cell lines

Mouse sarcoma S180 and hepatoma H22 cell lines were provided by Zhejiang Academy of Medical Sciences. Human leukemia K562 cell lines were purchased from Institute of Cell Biology, Chinese Academy Sciences. They were maintained in the logarithmic phase of growth in RPMI 1640 medium supplemented with 2 mM L-glutamine (Sigma), 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% fetal bovine serum at 37°C under humidified air with 5% CO2. S180 and H22 tumor cells were adapted to in vitro culture in RPMI 1640 complete medium. Tumor cells were washed and resuspended at 105 cells/ml in PBS. The cells were lysed by five freeze/thaw cycles in liquid nitrogen and then in water. The mixture was centrifuged at 17,000 × g at 4°C for 15 min and the supernatant (lysate) was collected, filtered through a 0.22-μm Millicel filter and frozen at −80°C. Protein concentration in lysates was determined by the Bradford assay (Stoscheck, 1990).

2.4. Extraction, isolation and purification of polysaccharides

The roots of Actinidia eriantha were collected in Wuyi county, Zhejiang province, China in August 2007, and identified by professor Xiang-Jie Xue at College of Pharmaceutical Sciences, Zhejiang University. A voucher specimen (No. 2007070806) has been deposited at the Laboratory of Nature Drug, College of Animal Sciences, Zhejiang University, China. The plant material (1 kg) was extracted with boiling water three times under reflux. The aqueous extract was filtered through Whatman filter paper. The filtrate was concentrated in a rotary evaporator under reduced pressure, and then centrifuged at 3000 rpm for 15 min. The supernatant was precipitated with three volumes of 95% ethanol, and stored overnight at 4°C. The precipitate was collected by vacuum filtration in bush funnel, and then washed successively with ethanol, acetone and petroleum ether to defat. The resulting precipitate was dissolved in distilled water and dialyzed against distilled water (cut-off MW 7000 Da). The retentate portion was concentrated under a reduced pressure and lyophilized to afford crude Actinidia eriantha polysaccharide (CAEP, 49.4 g). The crude polysaccharide was subjected to DEAE-Sephadex A-50 column chromatography, washed with H2O and eluted with 1.0 M NaCl solution. Most of the pigments were absorbed in the column. The eluates were concentrated under reduced pressure to an appropriate volume, and then dialyzed against distilled water. The retentate portion was lyophilized to afford a total Actinidia eriantha polysaccharide (AEP, light off-white powder, 34.7 g). Meanwhile, CAEP was dissolved in 0.1 M NaCl solution and through filter paper (0.45 μm). The filtrate was subjected to DEAE-Sephadex A-50 column chromatography and eluted with NaCl gradients (0.1–2.0 M). The eluate (5 ml) was collected and monitored for carbohydrate content using phenol–sulfuric acid method (Dubois et al., 1956). According to the elution profile, the eluates were combined, concentrated, dialyzed and lyophilized to give four fractions. These fractions were further chromatographed on a Sephacryl S-400 gel filtration column with water and lyophilized to yield four white polysaccharides, namely AEP-A, AEP-B, AEP-C, and AEP-D. AEP and four purified polysaccharides were endotoxin free with Limulus amebocyte lysate (LAL) test.

2.5. Molecular weight and composition analysis of polysaccharides

Total sugar content was estimated using the phenol–sulfuric acid analysis using glucose as standard (Dubois et al., 1956). Uronic acid content was determined by the carbazole–sulfuric acid method using glucuronic acid as standard (Bitter and Muir, 1962). The average molecular weight was determined by the HPGPC, which was performed on a Waters HPLC system (Alliances 2695, Waters, USA) equipped with a Waters Ultrahydrogel 250 column (7.8 mm × 300 mm) and a Waters 2410 differential refractometer. The mobile phase was 0.1 M l/1 NaNO3, and the flow rate was 0.9 ml/min. The sample (2 μg) was dissolved in the mobile phase
were seeded into 4 wells of a 96-well flat-bottom microtiter plate at 5 × 10^4 cell/ml in 100 μl complete medium, thereafter Con A (final concentration 5 μg/ml), LPS (final concentration 10 μg/ml), or RPMI1640 medium were added giving a final volume of 200 μl. The plates were incubated at 37 °C in a humid atmosphere with 5% CO2. After 44 h, 50 μl of MTT solution (2 mg/ml) were added to each well and incubated for 4 h. The plates were centrifuged (1400 × g, 5 min) and the untransformed MTT was carefully removed by pipetting. To each well 150 μl of a DMSO working solution (180 μl DMSO with 20 μl 1N HCl) was added, and the absorbance was evaluated in an ELISA reader (Bio-Rad, USA) at 570 nm after 15 min. The stimulation index (SI) was calculated based on the following formula: SI, the absorbance value for mitogen-cultures divided by the absorbance value for non-stimulated cultures.

2.8. Assay of natural killer (NK) cell activity

The activity of NK cells was measured as previously described (Tu et al., 2008). Briefly, K562 cells were used as target cells and seeded in 96-well U-bottom microtiter plate at 2 × 10^4 cells/well in RPMI 1640 complete medium. Splenocytes prepared as described above were used as the effector cells, and were added at 1 × 10^6 cells/well to give E/T ratio 50:1. The plates were then incubated for 20 h at 37 °C in 5% CO2 atmosphere. 50 μl of MTT solution (2 mg/ml) was added to each well and the plate was incubated for another 4 h and subjected to MTT assay. Three kinds of control measurements were performed: target cells control, blank control and effector cells control. NK cell activity was calculated as following equation: NK activity (%) = (ODT - ODS)/(ODT - OD2) × 100%, where OD1, optical density value of target cells control, OD2, optical density value of test samples, OD3, optical density value of effector cells control.

2.9. Assays of cytotoxic T lymphocyte (CTL) activity

The CTL activity was analyzed using MTT method as described above. Tumor (S180 or H22) cells and splenocytes were used as target cells and effector cells, respectively. The ratio of effector cells to target cells was 50:1. To determine the percentage of target cells killed, the following equation was used: % lysis = (ODT - OD3 - OD2)/OD2 × 100, where OD1, optical density value of target cells control, OD2, optical density value of test samples, OD3, optical density value of effector cells control.

2.10. Cytokine levels in the supernatants of cultured splenocytes

Splenocytes (5 × 10^6 cells/well) from tumor-bearing mice were prepared as described before were incubated with Con A (final concentration 5 μg/ml) in 24-well culture plates at 37 °C in 5% CO2. After 48 h, the plate was centrifuged at 1400 × g for 5 min and the supernatants were collected for the detection of IL-2 and IFN-γ levels using commercial ELISA kits. Briefly, culture supernatants or cytokine standards were added to 96-well flat-bottom microtiter plates coated with coating antibody, and plates then incubated at 37 °C for 1.5 h (IL-2) or 2 h (IFN-γ). Plates were washed and a detecting antibody was added to each well. Plates were incubated at 37 °C for 1 hour before addition of avidin–biotin–peroxidase complex (ABC). After incubation for 30 min, plates were washed and developed with tetramethyl benzidine (TMB) at 37 °C for 15 min. The reaction was stopped by addition of 100 μl of stop solution. The absorbance was measured in an ELISA reader at 450 nm.

2.11. Measurement of antigen-specific antibody

The antigen-specific IgG, IgG1, IgG2a and IgG2b antibodies in sera were detected by an indirect ELISA as previously described (Tu et al., 2008). Splenocytes were collected from sacrificed mice under aseptic conditions, in Hank’s balanced salt solution (HBSS, Sigma), was minced using a pair of scissors and passed through a fine steel mesh to obtain a homogeneous cell suspension, and the erythrocytes were lysed with ammonium chloride (0.8%, w/v). After centrifugation (380 × g at 4 °C for 10 min), the pelleted cells were washed three times in PBS and resuspended in RPMI 1640 complete medium. Cell numbers were counted with a haemocytometer by trypan blue dye exclusion technique. Cell viability exceeded 95%. Splenocyte proliferation was assayed as previously described (Tu et al., 2008). Splenocytes were prepared as described before were incubated with Con A (final concentration 5 μg/ml) in 24-well culture plates at 37 °C in 5% CO2. After 48 h, the plate was centrifuged at 1400 × g for 5 min and the supernatants were collected for the detection of IL-2 and IFN-γ levels using commercial ELISA kits. Briefly, culture supernatants or cytokine standards were added to 96-well flat-bottom microtiter plates coated with coating antibody, and plates then incubated at 37 °C for 1.5 h (IL-2) or 2 h (IFN-γ). Plates were washed and a detecting antibody was added to each well. Plates were incubated at 37 °C for 1 hour before addition of avidin–biotin–peroxidase complex (ABC). After incubation for 30 min, plates were washed and developed with tetramethyl benzidine (TMB) at 37 °C for 15 min. The reaction was stopped by addition of 100 μl of stop solution. The absorbance was measured in an ELISA reader at 450 nm.
In brief, microtiter plate wells were coated with 100 μl of the tumor cell lysate (protein concentration 50 μg/ml, in 50 mM carbonate–bicarbonate buffer, pH 9.6) for 24 h at 4 °C. The wells were washed three times with PBS containing 0.05% (v/v) Tween 20 (PBS/Tween), and then blocked with 5% FCS/PBS at 37 °C for 2 h. After three washings, 100 μl of diluted sera sample or 0.5% FCS/PBS as control were added to triplicate wells. The plates were then incubated for 2 h at 37 °C, followed by 3 times of washing. Aliquots of 100 μl of rabbit anti-mouse IgG horseradish peroxidase conjugate diluted 1:16,000, goat anti-mouse IgG1 peroxidase conjugate 1:8000, IgG2a peroxidase conjugate 1:8000, and IgG2b peroxidase conjugate 1:8000 with 0.5% FCS/PBS were added to each plate. The plates were further incubated for 2 h at 37 °C. After washing, the peroxidase activity was assayed as follows: 100 μl of substrate solution (10 mg of O-phenylendiamine and 37.5 μl of 30% H2O2 in 25 ml of 0.1 M citrate–phosphate buffer, pH 5.0) was added to each well. The plate was incubated for 10 min at 37 °C, and enzyme reaction was terminated by adding 50 μl/well of 2N H2SO4. The optical density was measured in an ELISA reader at 490 nm, where sets of sera samples have been subjected to within and between group comparisons, ELISA assays were performed on the same day for all of the samples.

2.12. Statistical analysis

The data were expressed as mean ± standard deviation (S.D.) and examined for their statistical significance of difference with ANOVA and a Tukey post hoc test. P-values of less than 0.05 were considered to be statistically significant.

3. Result

3.1. Composition and characterization of polysaccharides

The yield of the total polysaccharide AEP was 3.47% of the plant raw material, and the yields of four purified polysaccharides were 35.07%, 13.73%, 15.15% and 6.32% for AEPA, AEPB, AEPC and AEPD, respectively. In addition, no signs of toxicity were observed in the mice treated with these polysaccharides.

3.2. Inhibition of polysaccharides on the growth of transplanted tumors in mice

The inhibitory effect of AEP and four purified polysaccharides on the growth of tumor transplanted in mice was shown in Tables 1 and 2. As a positive control, CTX showed high inhibitory rate on S180 sarcoma and H22 hepatoma transplanted in mice. The growth of transplanted S180 sarcoma in mice was significantly inhibited by AEP in dose-dependent manner compared with the model controls (P < 0.001), with the inhibitory rate being 47.81, 50.11 and 55.34% at the doses of 2.5, 5.0 and 10 mg/kg, respectively. AEP similarly inhibited the growth of transplantable H22 hepatoma in mice, leading to a significant tumor regression (data not shown). Four purified polysaccharides also significantly inhibited the growth of H22 hepatoma transplanted in mice (P < 0.001), with the inhibitory rate being 48.61, 47.11, 56.67, and 54.05 for AEPA, AEPB, AEPD and AEPD, respectively. In addition, no signs of toxicity were observed in the mice treated with these polysaccharides on the basis of body weight (Tables 1 and 2) and microscopic examination of individual organs (intestinal tract, liver and kidney).

3.3. Effect of polysaccharides on splenocyte proliferation in tumor-bearing mice

The effect of AEP and four purified polysaccharides on mitogen-stimulated splenocyte proliferation in tumor-bearing mice was shown in Figs. 1 and 2. Con A- and LPS-induced splenocyte proliferation in the S180-bearing mice was significantly enhanced by AEP at the doses of 5.0 and 10 mg/kg (P < 0.001). Four purified polysaccharides also significantly promoted Con A- and LPS-stimulated splenocyte proliferation in the H22-bearing mice at the tested dose (P < 0.01 or P < 0.001). However, Con A- and LPS-stimulated splenocyte proliferations in the CTX-treated group were significantly lower than those of the model control (P < 0.05 or P < 0.01). Our experiment results also proved that immune function of tumorbearing mice decreased compared with normal mice (P < 0.001).

3.4. Effect of polysaccharides on the activity of NK cells and CTL in tumor-bearing mice

Tumor cell elimination is known to be mediated in part by the cytotoxic activity of NK cells and CTL. We therefore measured the cytotoxic activity of splenocytes against tumor cell and NK cell-sensitive K562 cells, and the results were shown in Figs. 3 and 4. AEP could significantly increase the activity of NK cells and CTL in the S180-bearing mice at three doses (P < 0.01 or P < 0.001), especially at the dose of 5.0 mg/kg. The activities of NK cells and CTL in the H22-bearing mice were also significantly enhanced by four purified polysaccharides (P < 0.01 or P < 0.001). However, NK cell and CTL activities in the CTX-treated mice were significantly lower than those of the model control (P < 0.01 or P < 0.001).
Table 1
Inhibitory effect of the total polysaccharide from the roots of *Actinidia eriantha* (AEP) on the growth of S180 sarcoma transplanted in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Weight (g) Before treatment</th>
<th>Weight (g) After treatment</th>
<th>Tumor weight (g)</th>
<th>Inhibitory rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>–</td>
<td>22.64 ± 1.11</td>
<td>30.09 ± 2.98</td>
<td>2.33 ± 0.27</td>
<td>–</td>
</tr>
<tr>
<td>CTX</td>
<td>20</td>
<td>22.10 ± 0.87</td>
<td>27.28 ± 1.34</td>
<td>0.76 ± 0.06</td>
<td>67.15</td>
</tr>
<tr>
<td>AEP</td>
<td>2.5</td>
<td>22.30 ± 1.03</td>
<td>29.90 ± 2.08</td>
<td>1.21 ± 0.20</td>
<td>47.81</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>22.07 ± 0.93</td>
<td>28.94 ± 2.54</td>
<td>1.16 ± 0.11</td>
<td>50.11</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>21.99 ± 0.75</td>
<td>28.27 ± 1.98</td>
<td>1.04 ± 0.18</td>
<td>55.34</td>
</tr>
</tbody>
</table>

The values are presented as means ± S.D. (*n* = 10). Significant differences with model control (MC) were designated as *P* < 0.05 and *P* < 0.001. CTX: cyclophosphamide (positive control).

Table 2
Inhibitory effect of four purified polysaccharides from the roots of *Actinidia eriantha* on the growth of H22 hepatoma transplanted in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Weight (g) Before treatment</th>
<th>Weight (g) After treatment</th>
<th>Tumor weight (g)</th>
<th>Inhibitory rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>–</td>
<td>22.35 ± 1.31</td>
<td>33.41 ± 2.31</td>
<td>1.67 ± 0.24</td>
<td>–</td>
</tr>
<tr>
<td>CTX</td>
<td>40</td>
<td>21.66 ± 0.92</td>
<td>30.68 ± 1.98</td>
<td>0.35 ± 0.10</td>
<td>86.75</td>
</tr>
<tr>
<td>AEPB</td>
<td>10</td>
<td>21.83 ± 0.94</td>
<td>32.00 ± 2.21</td>
<td>0.93 ± 0.32</td>
<td>48.61</td>
</tr>
<tr>
<td>AEPB</td>
<td>10</td>
<td>21.58 ± 0.86</td>
<td>32.27 ± 2.27</td>
<td>0.95 ± 0.37</td>
<td>47.11</td>
</tr>
<tr>
<td>AEPB</td>
<td>10</td>
<td>21.73 ± 1.14</td>
<td>33.51 ± 1.90</td>
<td>0.81 ± 0.32</td>
<td>56.67</td>
</tr>
<tr>
<td>AEPB</td>
<td>10</td>
<td>21.60 ± 1.45</td>
<td>33.33 ± 2.89</td>
<td>0.85 ± 0.24</td>
<td>54.05</td>
</tr>
</tbody>
</table>

The values are presented as means ± S.D. (*n* = 10). Significant differences with model control group (MC) were designated as *P* < 0.05 and *P* < 0.001. CTX: cyclophosphamide (positive control).
3.5. Effect of polysaccharides on cytokine secretion from splenocytes in tumor-bearing mice

Since cytokines play a prominent role in the development of immune response, we investigated the effect of four purified polysaccharides on the production of cytokines IL-2 and IFN-γ from Con A-stimulated splenocytes in H22-bearing mice by ELISA. As shown in Fig. 5, the production of IL-2 and IFN-γ was significantly decreased in CTX-treated mice as compared with model control (P<0.001). Four purified polysaccharides, especially AEPC and AEPD, markedly augmented IL-2 and IFN-γ production from splenocytes in H22-bearing mice (P<0.001).

3.6. Effect of polysaccharides on humoral immune responses

AEP and four purified polysaccharides were also assessed for their immunopotential potentials by the level of serum tumor antigen-specific IgG, IgG1, IgG2a, and IgG2b antibody in tumor-bearing mice. As shown in Fig. 6, the serum specific IgG, IgG2a and IgG2b antibody levels in S180-bearing mice were significantly enhanced by AEP at the three doses (P<0.05, P<0.01 or P<0.001). Significant enhancements in total serum specific IgG, IgG2a and IgG2b antibody levels were also observed in the H22-bearing mice treated with four purified polysaccharides except for AEPA compared with model control group (P<0.05, or P<0.01) (Fig. 7). The lower serum IgG1 antibody levels were found in the tumor-bearing mice. There were, however, no significant differences in the IgG1 antibody response between tumor-bearing mice treated and untreated. In this experiment, it was also found that CTX significantly decreased the serum IgG, IgG2a, and IgG2b antibody levels in S180-bearing mice (P<0.01).

4. Discussion

The roots of *Actinidia eriantha* have been used for cancers in the Chinese folk medicine, and were proved to have antitumor and immunopotentiating activities (Lin et al., 1987). In this investigation, we have succeeded in isolating the total polysaccharide AEP and four purified polysaccharides from the roots of *Actinidia eriantha*, and the in vivo therapeutic efficacies of these polysaccharides on the growth of mouse transplantable S180 sarcoma and H22 hepatoma were evaluated. AEP and four purified polysaccharides could significantly inhibit the growth of tumor transplanted in mice compared with model controls (Tables 1 and 2). Among four purified polysaccharides, AEP and AEPD exhibited higher antitumor effect than AEPA and AEPB, while there were no significant differences.

The immune system plays an important role in antitumor defense. Many reports suggested that the antitumor activity of the polysaccharides from several traditional Chinese herbs was also mediated through augmentation of the immune response (Wasser, 2002; Lee and Jeon, 2005; Schepetkin and Quinn, 2006; Cho and Leung, 2007). Therefore, we further investigated the effect of these polysaccharides on the cellular and humoral immunity in tumor-bearing mice to analyze the underlying mechanism of their antitumor activity. The humoral immune response by B-cells is an antigen-specific antibody reaction. Cell-mediated immune defense was mediated specifically by T-cells including NK cells. In addition to killing the tumor cells directly, T-cells can produce many

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**Fig. 5.** Effect of four purified polysaccharides from the roots of *Actinidia eriantha* on cytokine production from splenocytes in H22-bearing mice. Splenocytes were prepared and cultured with Con A for 48 h. The contents of IL-2 and IFN-γ in the culture supernatants were determined by ELISA as described in the text. The values are presented as means ± S.D. (n = 8). Significant differences compared to model control group (MC) are designated as a P<0.001, b P<0.01, or c P<0.05. CTX: cyclophosphamide (positive control).

**Fig. 6.** Effect of the total polysaccharide from the roots of *Actinidia eriantha* (AEP) on tumor antigen-specific IgG, IgG2a and IgG2b antibody levels in S180-bearing mice. The serum antigen-specific IgG, IgG2a and IgG2b antibodies were measured by ELISA as described in the text. The values are presented as means ± S.D. (n = 5). Significant differences compared to model control group (MC) are designated as a P<0.05, b P<0.01 and c P<0.001. CTX: cyclophosphamide (positive control).

**Fig. 7.** Effect of four purified polysaccharides from the roots of *Actinidia eriantha* on tumor antigen-specific IgG, IgG2a, and IgG2b antibody levels in H22-bearing mice. The serum antigen-specific IgG, IgG2a, and IgG2b antibodies were measured by ELISA as described in the text. The values are presented as means ± S.D. (n = 8). Significant differences compared to model control group (MC) are designated as a P<0.05 and b P<0.01. CTX: cyclophosphamide (positive control).
lymphocyte factors consisting of macrophage mobile factor, lymphotixin, transfer factor and interferon. Such factors could promote the proliferation and differentiation of immune cells, macrophage phagocytosis and the capacity of killing target cells, so that they play a role in preventing tumor (Kim et al., 2001). The capacity to elicit an effective T- and B-cell immunity can be shown by the stimulation of lymphocyte proliferation response (Marciani et al., 2000). It is generally known that Con A stimulates T-cells and LPS stimulates B-cell proliferation. The proliferation assay showed that AEP and four purified polysaccharides could significantly promote the Con A- and LPS-stimulated splenocyte proliferation in tumor-bearing mice, while the positive control CTX with high tumor inhibitory rate had immunosuppressive effect on splenocyte proliferation (Figs. 1 and 2). The results indicated that these polysaccharides could significantly increase the activation potential of T- and B-cells and enhance the humoral immunity and cell-mediated immunity in tumor-bearing mice.

NK cells and CTL represent two major populations of cytotoxic lymphocytes (Kos and Engleman, 1996; Medzhitov and Janeway, 1997), and are important in the defense against tumors and viruses (Boon et al., 1994; Moretta et al., 2001). NK cells and CTLs are able to kill autologous cells infected with intracellular pathogens, as well as tumor cells. NK cells are functionally similar to CTLs. Unlike CTLs, however, the killing by NK cells is non-specific, and NK cells do not need to recognize antigen/MHC on the target cell. NK cells can react against and destroy target cell without prior sensitization to it. NK cell activity assay is a routine method for analysis of a patient's cellular immune response in vitro, and can also be used to test the antitumor activities of possible drugs (Zhang et al., 2005). In this study, AEP and four purified polysaccharides were found to significantly enhance the killing activity of NK cells and CTL from splenocytes in tumor-bearing mice (Figs. 3 and 4), suggesting that these polysaccharides could enhance the specific and non-specific cytolytic activities against autologous tumor cells.

Cytokines regulate both cellular and humoral immune responses by affecting immune cell proliferation, differentiation and functions. IL-2 has many immunopotentiating effects, such as proliferation of T-cells, B-cells, NK cells and monocytes, augmentation of cytotoxicities of T-cells and NK cells and in vivo generation of lymphokine-activated killer (LAK) cells, which exhibit high cytolytic activities against autologous tumor cells (Asano et al., 1997). IFN-γ is one of the major important immunoregulatory molecule with antitumor and immunomodulatory properties (Blankenstein and Qin, 2003). IFN-γ could inhibit cell proliferation and angiogenesis in the tumor microenvironment (Boehm et al., 1997). In the present study, the levels of IL-2 and IFN-γ secreted by splenocytes in H22-bearing mice were significantly increased by four purified polysaccharides (Fig. 5). The increase may also explain the antitumor properties of these polysaccharides.

Some antibodies can act either directly by blocking signal transduction pathways (i.e. when targeted to growth factor receptors) or indirectly via the activation of NK-mediated killing (ADCC; antibody-dependent cellular cytotoxicity) (Melero et al., 2007; Nimmerjahn and Ravetch, 2007). To investigate the effect of AEP and four purified polysaccharides on the humoral immune responses in tumor-bearing mice, the tumor antigen-specific antibody levels in serum were measured using ELISA. As shown in Figs. 6 and 7, AEP and four purified polysaccharides significantly not only increased the serum antigen-specific IgG antibody levels, but enhanced IgG2a and IgG2b antibody levels in tumor-bearing mice. This clearly demonstrated that these polysaccharides modulated the quality of immune responses, and mainly proved a Th1 type of immune response in tumor-bearing mice as associated sensitively with an enhancement of IgG2a and IgG2b levels (Finkelman et al., 1990; Germann et al., 1995). These results further confirmed that AEP and four purified polysaccharides could promote the specific humoral immune response.

In this study, four purified polysaccharides were found to contain similar neutral monosaccharide composition. However, the composition and the ratio of these monosaccharides greatly differed from one another. There also were significant differences in the average molecular weights, as well as the contents of glucose and uronic acid among four polysaccharides. Based on both the chemical differences observed and the different potencies in the pharmacological activities among four purified polysaccharides, it was presumed that the composition and the ratio of monosaccharides, the contents of glucose and uronic acid, as well as molecular weight could affect their antitumor and immunomodulatory activity.

In conclusion, AEP and four purified polysaccharides from the roots of Actinidia eriantha could not only significantly inhibit the growth of transplantable tumor in mice, but also remarkably increase splenocytes proliferation, NK cell and CTL activity, the level of the cytokines secreted by splenocytes, and serum antigen-specific antibody levels in tumor-bearing mice, which indicated that the AEP and four purified polysaccharides could improve both specific and non-specific cellular and humoral immune response. The above results suggested that the antitumor activity of these polysaccharides might be achieved by improving immune response, and they could act as antitumor agent with immunomodulatory activity.

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References


