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

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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 fours 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 splenocytes 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.

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, lentinan, schizophyllan and krestin have been accepted as immunoceuticals 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 possessed the antitumor and immunopotentiating activities (Lin et al., 1987).

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Some triterpenes, such as ursolic acid, 2α,3α,24-trihydroxyurs-12-en-28-oic, 24-acetyloxy-2α,3α-dihydroxyurs- 2-en-28-oic acid (eriatic acid A), 2β,3β,23-trihydroxyurs-12-en-28-oic acid (eriantic acid B), 2α,3β-dihydroxyurs- 12-en-28-oic acid, 2β,3β-dihydroxy-23-oxours-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 chromatograph, 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 AEPA, AEPB, AEPC and AEPD, 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, trifluoroacetic 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 Sephacryl S-400 were from Amersham Biosciences Co., Piscataway, NJ, USA; RPMI-1640 medium and fetal calf serum (FCS) were from Gibco, 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 Boster 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% CO_2. S180 and H22 tumor cells were adapted to *in vitro* culture in RPMI 1640 complete medium. Tumor cells were washed and resuspended at 10^7 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 Millipore 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-Ji Xue at College of Pharmaceutical Sciences, Zhejiang University. A voucher specimen (No. 20070806) 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 büshi funnel, and then washed sequentially with ethanol, acetone and petroleum ether to defat. The resulting precipitate was dissolved in distilled water and dialyzed against distilled water (cut-off M_w 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 H_2O, and eluted with 1.0 M NaCl solution. Most of the pigments were absorbed in the column. The eluates collected were concentrated under reduced pressure to an appropriate volume, and then dialysed 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 chromatographied on a Sephacryl S-400 gel filtration column with water and lyophilized to yield four white polysaccharides, namely AEPA, AEPB, AEPC and AEPD. 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 by 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 (Allances 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 mol/l $NaNO_3$, and the flow rate was 0.9 ml/min. The sample (2 mg) was dissolved in the mobile phase

(0.2 ml) and centrifuged. A 20 μl sample was injected in each run. The molecular weight was estimated by reference to the calibration curve made from a Dextran T-series standard of known molecular weight (6100, 16,500, 26,290, 40,000, 84,000, 158,000) (Sun et al., 2005). Neutral monosaccharide composition was analyzed according to the following procedure: the polysaccharide samples (5 mg) were hydrolyzed with 5 ml of 2 M TFA at 110 °C for 5 h to release component monosaccharides. Then the hydrolyzed monosaccharides (inositol as the internal standard) were derivatized to acetylated aldononitriles (Mawhinney et al., 1980) and isothermally separated by gas chromatography (GC) on an Agilent 4890D system (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector (FID) and a DB-35 capillary column (30.0 m × 0.32 nm × 0.25 μm). N_2 was used as the carrier gas (1.2 ml/min). The injector temperature was kept at 250 °C (split injection 40:1). The operation was performed at a column temperature program from 110 °C to 190 °C at 5 °C/min, holding for 5 min at 190 °C, then increasing to 250 °C at 10 °C/min and finally holding for 5 min at 250 °C. The molar percentage of the component monosaccharides was calculated as follows. The correction factor is shown in the equation: $f_{i/s} = (W_i/W_s)/(A_i/A_s)$, where A_s and A_i are the values of peak areas for inositol and standard monosaccharide, respectively. W_s and W_i are the values of weights for inositol and standard monosaccharide, respectively. The molar ratio value is shown in the equation: $R_{i/s} = f_{i/s} \times (A_i/A_s)/M$, where A_i/A_s is the ratio value of peak area for the component monosaccharide of tested samples and inositol. M is the molecular weight of the monosaccharides and $f_{i/s}$ is the correction factor.

2.6. Treatment and drug administration

Ascites of the tumor-bearing mice were drawn out under aseptic conditions and then diluted 4-fold with aseptic saline. The diluted tumor cell suspension was inoculated subcutaneously (s.c.) into the armpit for 0.2 ml per mouse (Tu et al., 2008). 24 h later, mice were divided into five or six groups, each consisting of ten mice. In experiment I, the mice inoculated with S180 cells were orally administered with AEP in saline at the doses of 2.5, 5.0, and 10.0 mg/kg for 10 days or injected intraperitoneally (i.p.) with CTX at a dose of 20 mg/kg every other day for 5 times. In experiment II, the mice inoculated with H22 cell were orally administered with AEPA, AEPB, AEPC and AEPD in saline at a single dose of 10 mg/kg for 10 days or injected i.p. with CTX at a dose of 40 mg/kg for 2 day once daily. In both experiments, the model control group received the same volume of saline. The dose volume was 0.2 ml/10 g body weight. A normal control group without medicine administration and tumor inoculation was also used in both experiments. On day 11, mice were weighed, and sacrificed by cervical dislocation. The solid tumors were collected and weighed. Then the inhibitory rate against the growth of tumor was calculated. The inhibitory rate (%) = $[(C - T)/C] \times 100$, where C is the average tumor weight of the model control group; T is the average tumor weight of treated groups.

2.7. Splenocyte proliferation assay

Spleen 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 seeded into 4 wells of a 96-well flat-bottom microtiter plate at 5×10^6 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% CO_2. 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% CO_2 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 (%) = $(OD_T - (OD_S - OD_E))/OD_T \times 100\%$, where OD_T, optical density value of target cells control, OD_S, optical density value of test samples, OD_E, 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 = $(OD_T - (OD_S - OD_E))/OD_T \times 100$, where OD_T, optical density value of target cells control, OD_S, optical density value of test samples, OD_E, optical density value of effector cells control.

2.10. Cytokine levels in the supernatants of cultured splenocytes

Splenocytes (5×10^5 cells/well) from tumor-bearing mice prepared as described before were incubated with Con A (final concentration 5 μg/ml) in 24-well culture plates at 37 °C in 5% CO_2. 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

(Sun et al., 2004). In brief, microtiter plate wells were coated with 100 μ

Table 1
Inhibitory effect of the total polysaccharide from the roots of *Actinidia eriantha* (AEP) on the growth of S180 sarcoma transplanted in mice.

Group	Dose (mg/kg)	Weight (g)		Tumor weight (g)	Inhibitory rate (%)
		Before treatment	After treatment		
MC	–	22.64 ± 1.11	30.09 ± 2.98	2.33 ± 0.27	–
CTX	20	22.10 ± 0.87	27.28 ± 1.34[a]	0.76 ± 0.08[c]	67.15
AEP	2.5	22.30 ± 1.03	29.90 ± 2.08	1.21 ± 0.20[c]	47.81
	5.0	22.07 ± 0.93	28.94 ± 2.54	1.16 ± 0.11[c]	50.11
	10	21.99 ± 0.75	28.27 ± 1.98	1.04 ± 0.18[c]	55.34

The values are presented as means ± S.D. ($n = 10$). Significant differences with model control (MC) were designated as [a]$P < 0.05$ and [c]$P < 0.001$. 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.

Group	Dose (mg/kg)	Body weight (g)		Tumor weight (g)	Inhibitory rate (%)
		Before treatment	After treatment		
MC	–	22.35 ± 1.31	33.41 ± 2.31	1.67 ± 0.24	–
CTX	40	21.66 ± 0.92	30.68 ± 1.98[a]	0.35 ± 0.10[c]	86.75
AEPA	10	21.83 ± 0.94	32.00 ± 2.21	0.93 ± 0.33[c]	48.61
AEPB	10	21.58 ± 0.86	32.27 ± 2.27	0.95 ± 0.37[c]	47.11
AEPC	10	21.73 ± 1.14	33.51 ± 1.90	0.81 ± 0.32[c]	56.67
AEPD	10	21.60 ± 1.45	33.33 ± 2.89	0.85 ± 0.24[c]	54.05

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

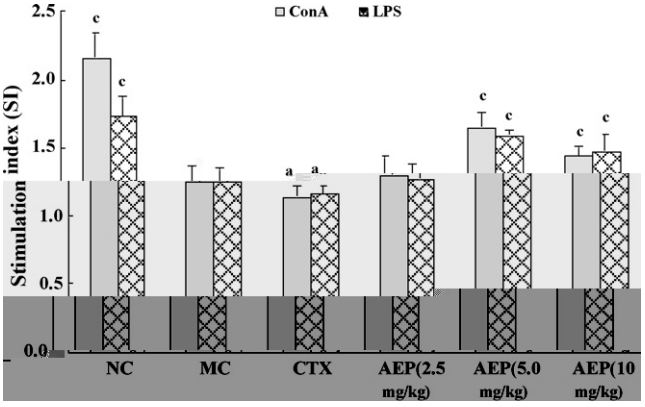

Fig. 1. Effect of the total polysaccharide from the roots of *Actinidia eriantha* (AEP) on Con A- and LPS-stimulated splenocyte proliferation in S180-bearing mice. Splenocyte proliferation was measured by the MTT method as described in the text, and shown as a stimulation index. The values are presented as means ± S.D. ($n = 5$). Significant differences compared to model control group (MC) are designated as [a]$P < 0.05$ and [c]$P < 0.001$. CTX: cyclophosphamide (positive control).

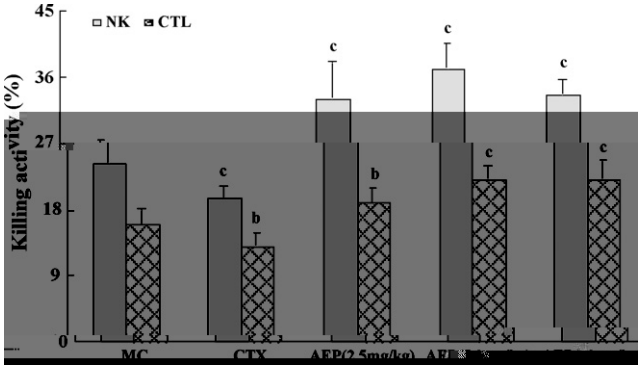

Fig. 3. Effect of the total polysaccharide from the roots of *Actinidia eriantha* (AEP) on NK cell and CTL activity in S180-bearing mice. Splenocytes were prepared and assayed for NK cell and CTL activity by the MTT method as described in the text. The values are presented as means ± S.D. ($n = 5$). Significant differences compared to model control group (MC) are designated as [b]$P < 0.01$ and [c]$P < 0.001$. CTX: cyclophosphamide (positive control).

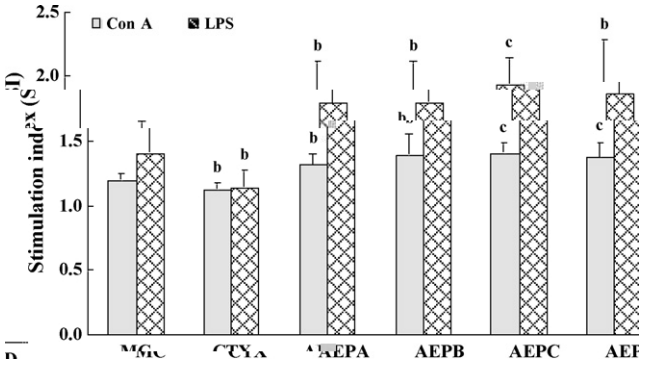

Fig. 2. Effect of four purified polysaccharides from the roots of *Actinidia eriantha* on Con A- and LPS-stimulated splenocyte proliferation in H22-bearing mice. Splenocyte proliferation was measured by the MTT method as described in the text, and shown as a stimulation index. The values are presented as means ± S.D. ($n = 8$). Significant differences compared to model control group (MC) are designated a [b]$P < 0.01$ and [c]$P < 0.001$. CTX: cyclophosphamide (positive control).

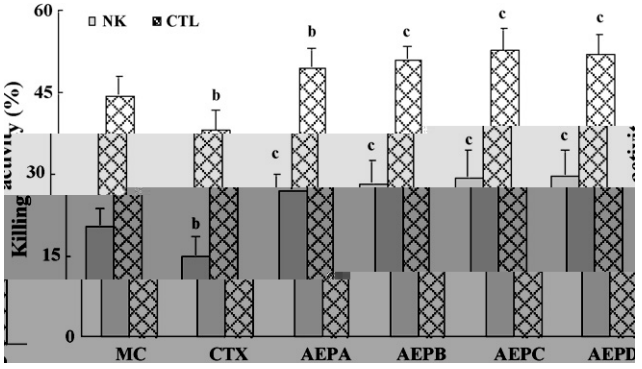

Fig. 4. Effect of four purified polysaccharides from the roots of *Actinidia eriantha* on NK cells and CTL activity in H22-bearing mice. Splenocytes were prepared and assayed for NK cell and CTL activity by the MTT method as described in the text. The values are presented as means ± S.D. ($n = 8$). Significant differences compared to model control group (MC) are designated as [b]$P < 0.01$ and [c]$P < 0.001$. CTX: cyclophosphamide (positive control).

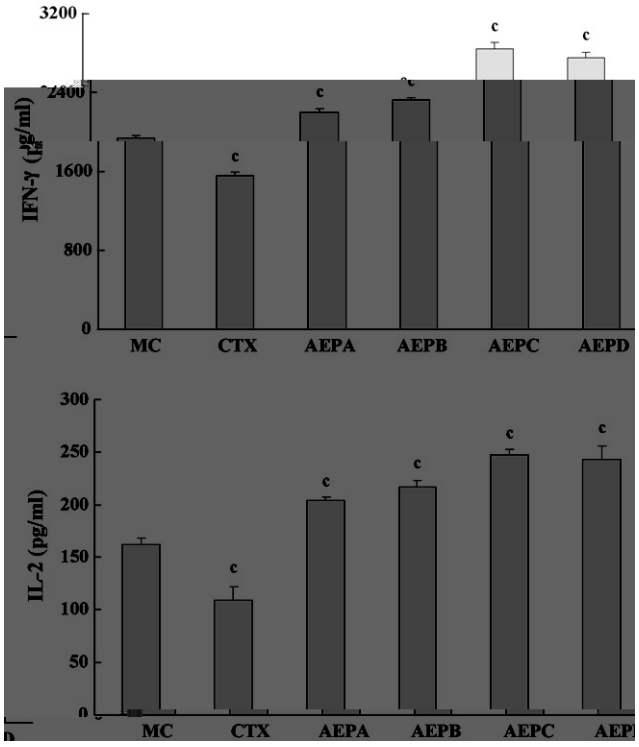

Fig. 5. Effect of four purified polysaccharides from the roots of *Actinidia eriantha* on cytokine production from splenocytes in H22-bearing mice. Splenocytes were prepared and cultured with Con A for 48 h. The contents of IL-2 and IFN-γ in the culture supernatants were determined by ELISA as described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

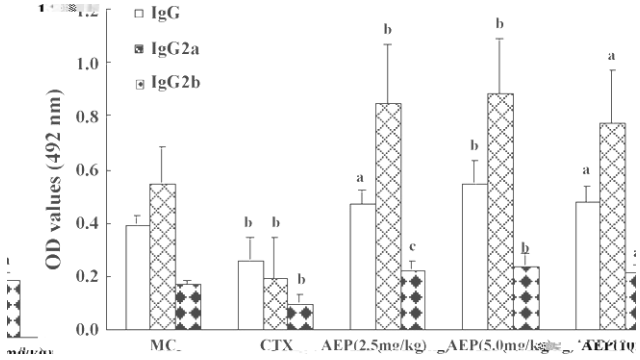

Fig. 6. Effect of the total polysaccharide from the roots of *Actinidia eriantha* (AEP) on tumor antigen-specific IgG, IgG2a and IgG2b antibody levels in S180-bearing mice. The serum antigen-specific IgG, IgG2a and IgG2b antibodies were measured by ELISA as described in the text. The values are presented 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).

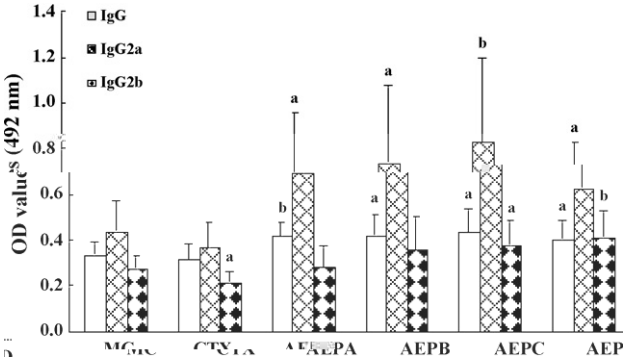

Fig. 7. Effect of four purified polysaccharides from the roots of *Actinidia eriantha* on tumor antigen-specific IgG, IgG2a, and IgG2b antibody levels in H22-bearing mice. The serum antigen-specific IgG, IgG2a, and IgG2b antibodies were measured by ELISA as described in the text. The values are presented 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).

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, AEPC 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

lymphocyte factors consisting of macrophage mobile factor, lymphotoxin, 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 cytotoxicites 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 (

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