

Preliminary report

## Immunomodulation and antitumor activity by a polysaccharide–protein complex from *Lycium barbarum*

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### Abstract

The modulation of a polysaccharide–protein complex from *Lycium barbarum* (LBP<sub>3p</sub>) on the immune system in S180-bearing mice was investigated. The mice inoculated with S180 cell suspension were treated p.o. with LBP<sub>3p</sub> (5, 10 and 20 mg/kg) for 10 days. The effects of LBP<sub>3p</sub> on transplantable tumors and macrophage phagocytosis, quantitative hemolysis of sheep red blood cells (QHS), lymphocyte proliferation, the activity of cytotoxic T lymphocyte (CTL), interleukin-2 (IL-2) gene expression and lipid peroxidation were studied. LBP<sub>3p</sub> could significantly inhibit the growth of transplantable sarcoma S180 and increase macrophage phagocytosis, the form of antibody secreted by spleen cells, spleen lymphocyte proliferation, CTL activity, IL-2 mRNA expression level and reduce the lipid peroxidation in S180-bearing mice. The effect is not dose-dependent in a linear fashion. A total of 10 mg/kg dose is more effective than 5 and 20 mg/kg doses. This suggests that LBP<sub>3p</sub> at 10 mg/kg has a highly significant effect on tumor weight and improves the immune system.

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**Keywords:** *Lycium barbarum*; Polysaccharide–protein complex; Transplantable tumor; Immune system

### 1. Introduction

During the past three decades, many polysaccharides and polysaccharide–protein complexes have been isolated from mushrooms, fungi, yeasts, algae, lichens and plants. The biological activities of these polysaccharides have attracted more attention recently in the biochemical and medical areas because of their

immunomodulatory and antitumor effects [1]. The search for novel polysaccharides with antitumor properties stems from the basic shortcomings of cancer chemotherapy and radiotherapy. In particular, the great majority of chemical compounds, which have been identified as cytotoxic to cancer cells, are also toxic to normal cells [2]. Hence, the discovery and identification of new safe drugs, without severe side effects, has become an important goal of research in the biomedical sciences. The enhancement or potentiation of host defense mechanisms has been recognized as a possible means of inhibiting tumor growth without harming the host.

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*Lycium barbarum* plays multiple roles in pharmacological and biological functions as a well-known Chinese traditional medicine and also a kind of food. *L. barbarum* polysaccharide–protein complex (LBP) is its important bioactive component. It was reported that the crude LBP could significantly suppress the growth of malignant tumor in vivo [3,4]. However, the antitumor effect of LBP is speculative because of the unclear purity of the crude LBP and the mechanism of antitumor action of LBP is still not fully understood. In the present work, the immunomodulative effect of LBP<sub>3p</sub>, the third fraction of LBP, on S180-bearing mice was investigated and the lipid peroxidation was also discussed.

## 2. Materials and methods

### 2.1. Preparation of LBP<sub>3p</sub>

*L. barbarum* was collected in Zhongning, Ningxia, China and identified by Prof. Qiong Luo, Medical School of Wuhan University, Wuhan, China. The voucher specimen of this plant (FST 505) is deposited in the Department of Food Science and Technology, Huazhong Agricultural University, Wuhan, China. A total of 100 g of dried powdered *L. barbarum* were first extracted with acetone/petroleum (1:1, 300 ml × 3) to remove pigments. The dried residual plant material was then extracted with 80% ethanol (300 ml × 3) to remove oligosaccharides. The pellets were extracted with distilled water (300 ml) at 80 °C every 2 h for four times, and the extracts were pooled and concentrated. The crude polysaccharide–protein complex (LBP) was precipitated by five volumes of ethanol. After centrifugation, the precipitate (3.35 g) was redissolved in distilled water (30 ml) and applied to DEAE-cellulose (OH<sup>-</sup>) anion exchange chromatography column (column size: 2.6 × 40 cm, 30 g). The column was eluted with H<sub>2</sub>O (480 ml), 0.05 M NaCl (500 ml), 0.10 M NaCl (500 ml) and 0.25 M NaCl (500 ml). A neutral and three acidic fractions were obtained, LBP<sub>1</sub> (0.535 g), LBP<sub>2</sub> (0.511 g), LBP<sub>3</sub> (0.659 g) and LBP<sub>4</sub> (1.333 g), respectively. Each fraction was dialyzed against distilled water for 3 days and then purified on Sephadex G200 column (column size: 2.6 × 60 cm, 10 g) with distilled water (200 ml) as the elutant. A purified fraction from LBP<sub>3</sub>

(LBP<sub>3p</sub>) was obtained after lyophilization with a yield of 0.297 g. It was identified to be homogeneous by SDS-PAGE [5], which showed a single band after staining with Ag [6] and periodic acid-schiff [7]. Chemical composition of LBP<sub>3p</sub> was 63.56% neutral sugars, 24.80% acidic sugars and 7.63% proteins as determined by phenol–H<sub>2</sub>SO<sub>4</sub> [8], carbazole [9] and Lowry method [10], respectively. It contained six monosaccharides: galactose, glucose, rhamnose, arabinose, mannose, xylose and the molar ratio was 1:2.12:1.25:1.10:1.95:1.76, respectively, by gas chromatography [11]. The molecular weight of LBP<sub>3p</sub> was  $1.57 \times 10^5$  by laser light scattering [12].

### 2.2. Experimental animals

Male Kunming mice (18 ± 2) g were provided by the Animal Center, Institute of Health and Epidemic Prevention, Wuhan, China. The mice were housed under normal laboratory conditions (21 ± 2 °C, 12/12-h light–dark cycle) with free access to standard rodent chow and water. Under sterile condition, 0.2 ml of S180 cell suspension (sterile normal saline 1:3 dilution) was inoculated subcutaneously to mouse armoit. The mice inoculated were divided into five groups: S180-bearing control group, 20 mg/kg cyclophosphamide (CTX) and 5, 10, 20 mg/kg LBP<sub>3p</sub> treatment groups. A normal control group was also used in this experiment. CTX and LBP<sub>3p</sub> were administered p.o. for 10 days once daily. Normal control and S180-bearing control groups received the same volume of normal saline. On day 11, all animals were executed. The mice, thymus and tumors were weighed, and a count on tumor inhibition rate was made. Thymus index was expressed as the thymus weight relative as body weight.

### 2.3. Macrophage phagocytosis assay

The mice were injected i.p. with 1.0-ml 6% starch broth 3 days prior to sacrifice. On day 11, the mice were injected i.p. with 1.0-ml 1% cock red blood cells (CRBC). After 30 min, the macrophages were collected, fixed with methanol and stained with Giemsa. The number of CRBC ingested by 100 macrophages were counted in an optical microscope and expressed as phagocytosis index (PI). Besides, the percentages of macrophages that phagocytosed CRBC were deter-

mined and expressed as phagocytic efficiency index (PEI).

#### 2.4. Quantitative hemolysis of sheep red blood cells (QHS) assay

QHS assay was performed using the methods of Simpson and Gozzo [13] with some modifications. In brief, 0.2 ml of 20% sheep red blood cells (SRBC) prepared in normal saline was injected to animals, i.p., 4 days prior to the assay. On day 4 following immunization, the spleens were removed and single cell suspensions of  $10 \times 10^6$ /ml were prepared in PBS. A total of 1.0 ml of 0.4% SRBC and 1.0 ml of 10% guinea pig serum were mixed with 1.0 ml of cell suspension and incubated for 1 h at 37 °C. After centrifugation at 3000 rpm for 3 min, the absorbance of the supernatant was measured at 413 nm using spectrophotometer (Shimadzu UV-1201).

#### 2.5. Lymphocyte proliferation assay

Spleens were aseptically removed from sacrificed mice with scissors and forceps in cold phosphate-buffered saline (PBS) and gently homogenized with a loose teflon pestle.  $5 \times 10^3$  cells were cultured in RPMI-1640 medium supplemented with 10% newborn bovine serum (NBS) at 37 °C in an atmosphere of 5% CO<sub>2</sub> in the presence of concanavalin A (ConA, 5 mg/l). After treatment for 72 h, 10 µl MTT (5 g/l) was added to every well and the plate was incubated for another 4 h. The plate was then centrifuged at 2000 rpm for 10 min and the supernatants were discarded. A total of 150 µl dimethyl sulfoxide was added to each well. The plate was then shaken until crystals were dissolved. The absorbance  $A_{570}$  was detected on the ELX800 Microplate Reader (BioTEK, USA) [14].

#### 2.6. Assays of cytotoxic T lymphocyte (CTL)

The CTL activity from spleen was studied using a Cytotox 96<sup>®</sup> Non-Radioactive Cytotoxicity Assay Kit (Promega). S180 cells were used as target cells and seeded in 96-well U-bottom culture plates at  $5 \times 10^4$  cells/well in RPMI-1640. Spleen cells were added at  $5 \times 10^5$  cells/well. The plates were centrifuged at 2000 rpm for 4 min to facilitate cell to cell contacts

and then they were incubated for 4 h at 37 °C. Lactate dehydrogenase (LDH) activity was measured in 50 µl/well of the supernatants by addition of the enzyme substrate and absorbance recording at 490 nm. Three kinds of control measurements were performed: a target spontaneous release, a target maximum release and an effector spontaneous release. To determine the percentage of target cells killed, the following equation was used: % lysis=(E – ES – TS)/(M – ES) × 100; where E=mean of absorbances in the presence of effector cells, ES=mean of absorbances of effector cells incubated alone, TS=mean of the absorbances of target cells incubated with medium alone, M=mean of maximum absorbances after incubating target cells with lysis solution.

#### 2.7. Interleukin-2 (IL-2) gene expression

Mice were sacrificed and their spleen were removed immediately, frozen in liquid nitrogen and then stored at –80 °C. Total RNA was extracted with TRIzol reagent (Gibco BRL) according to the manufacture's instruction and resuspended in 100 µl RNase-free water. Total RNA was quantified by using a spectrophotometer at 260 nm and the purity was assessed by determining the ratio of  $A_{260}/A_{280}$  (nm). Complementary DNA (cDNA) was prepared by incubation RNA with Molony murine leukemia virus (M-MLV) reverse transcriptase (200 unites, Promega), 10 mM dNTP (Roche) and Oligo (dT)<sub>15</sub> (200 ng, Promega) at 37 °C for 60 min in 25 µl Tris–HCl buffer (50 mM, pH 8.3). Following inactivation of the enzyme by incubation at 95 °C for 5 min, the cDNAs were amplified in a polymerase chain reaction (PCR) with the following primer sets: 5'-TGCAGCTCG-CATCCTGTGTCA-3' and 5'-ACTCCTCTACCTATCGGAAGA-3' for IL-2 (the product size 468 bp); 5'-TGGGTCAGAAGGACTCCTATG-3' and 5'-TCTTCTCGATACTC-GACGGAC-3' for β-actin as an internal control (product size: 591 bp). These sets were designed from the published nucleic acid sequences available from GenBank databases. PCR amplification was carried out with a reaction mixture composed of primers, dNTP and 1 unit Taq DNA polymerase (Promega). After heating samples at 95 °C for 5 min, 30 cycles of PCR were performed consisting of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min and extension at 72

Table 1  
Effects of LBP<sub>3p</sub> on thymus indexes tumor weights in S180-bearing mice

Group	Concentration (mg/kg)	Thymus index (mg/g)	Tumor weight (g)	Inhibitory rate (%)
Normal control	–	3.42 ± 0.67***	–	–
S180 control	–	2.01 ± 0.70	1.48 ± 0.32	–
CTX	20	2.23 ± 0.37	0.77 ± 0.30***	47.77
LBP <sub>3p</sub>	5	2.55 ± 0.15*	1.07 ± 0.39*	28.14
	10	2.80 ± 0.34**	0.84 ± 0.44***	43.05
	20	2.45 ± 0.30	1.31 ± 0.12*	11.88

S180-bearing mice were administered p.o. with CTX 20 mg/kg and LBP<sub>3p</sub> 5, 10, 20 mg/kg for 10 days once daily. Normal control and S180-bearing control groups received the same volume of normal saline. Thymus index, tumor weight and the inhibitory rate were determined on day 11. Values are mean ± S.D. of 10 mice.

\*Significantly different from S180 control group at  $p < 0.05$ .

\*\*Significantly different from S180 control group at  $p < 0.01$ .

\*\*\*Significantly different from S180 control group at  $p < 0.001$ .

°C for 1 min with a final extension at 72 °C for 5 min. An aliquot of PCR products was separated on a 1.0% agarose gel containing ethidium bromide and measured semiquantitatively using a Bio-Rad Multi-Analyst System. To compare the relative mRNA expression level from each of the samples, the value is presented as the ratio of the IL-2 band intensity of RT-PCR product over the corresponding  $\beta$ -actin RT-PCR product. The PCR products were sequenced to confirm the specificity of the oligonucleotide primers.

### 2.8. Lipid peroxidation assay

Ten percent homogenates of liver, spleen and serum were prepared in cold normal saline. After

centrifugation at 3000 rpm at 4 °C for 20 min, 1.0-ml 15% trichloroacetic acid (TCA) and 1.0-ml 6.7% thiobarbituric acid (TBA) were mixed with 1.0 ml of the supernatant and then boiled for 15 min. The contents were centrifuged at 3000 rpm for 10 min and the absorbance was measured at 532 nm. The content of protein was determined using Bradford's method. The concentration of TBARS was calculated using an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  [15].

### 2.9. Statistical analysis

Results were expressed as mean ± standard deviation (S.D.). The statistical significance of difference between groups was evaluated by analysis of vari-

Table 2  
Effects of LBP<sub>3p</sub> on macrophage phagocytosis and the form of antibody secreted by spleen cells in S180-bearing mice

Group	Concentration (mg/kg)	PEI (%)	PI	A <sub>413</sub>
Normal control	–	37.40 ± 3.58***	1.15 ± 0.09***	0.447 ± 0.012**
S180 control	–	28.40 ± 3.64	0.76 ± 0.05	0.340 ± 0.028
CTX	20	30.80 ± 2.28	0.81 ± 0.07	0.356 ± 0.021
LBP <sub>3p</sub>	5	33.20 ± 3.03*	0.90 ± 0.05**	0.390 ± 0.016**
	10	35.00 ± 3.67**	1.07 ± 0.07***	0.424 ± 0.014**
	20	31.60 ± 2.07	0.86 ± 0.09	0.381 ± 0.015*

S180-bearing mice were administered p.o. with CTX 20 mg/kg and LBP<sub>3p</sub> 5, 10, 20 mg/kg for 10 days once daily. Normal control and S180-bearing control groups received the same volume of normal saline. The phagocytosis index (PI), phagocytic efficiency index (PEI) and quantitative hemolysis of sheep red blood cells (QHS) were determined according to Section 2. Values are mean ± S.D. of 10 mice.

\*Significantly different from S180 control group at  $p < 0.05$ .

\*\*Significantly different from S180 control group at  $p < 0.01$ .

\*\*\*Significantly different from S180 control group at  $p < 0.001$ .

ance, followed by Student's *t*-test. A significant difference was set at  $p < 0.05$ .

### 3. Results

#### 3.1. Effects of LBP<sub>3p</sub> on thymus indexes and tumor weights in S180-bearing mice

As shown in Table 1, 5 and 10 mg/kg LBP<sub>3p</sub> caused a significant increase in the thymus index compared with S180 control group. No significant increase was observed in CTX-treated animals. LBP<sub>3p</sub> could significantly inhibit the growth of mouse transplantable sarcoma S180. The inhibitory rate was 28.14%, 43.05% and 11.88%, respectively, when the concentration of LBP<sub>3p</sub> was 5, 10 and 20 mg/kg. The inhibitory effect of LBP<sub>3p</sub> 10 mg/kg was the strongest and close to that of CTX 20 mg/kg, a positive control.

#### 3.2. Effects of LBP<sub>3p</sub> on macrophage phagocytosis and humoral immunity in S180-bearing mice

A significant reduction in macrophage phagocytosis and QHS assay was induced in S180-bearing mice. LBP<sub>3p</sub> could markedly increase macrophage phagocytosis and the antibody secreted by spleen cells in

Table 3  
Effects of LBP<sub>3p</sub> on spleen lymphocyte proliferation and CTL activity in S180-bearing mice

Group	Concentration (mg/kg)	Lymphocyte proliferation $A_{570}$	CTL activity (%)
Normal control	–	0.420 ± 0.010**	26.92 ± 2.92**
S180 control	–	0.233 ± 0.019	12.54 ± 0.49
CTX	20	0.251 ± 0.019	15.22 ± 0.85*
LBP <sub>3p</sub>	5	0.364 ± 0.019**	19.44 ± 2.32**
	10	0.397 ± 0.014***	23.82 ± 1.90**
	20	0.348 ± 0.019**	17.92 ± 2.16**

S180-bearing mice were administered p.o. with CTX 20 mg/kg and LBP<sub>3p</sub> 5, 10, 20 mg/kg for 10 days once daily. Normal control and S180-bearing control groups received the same volume of normal saline. The lymphocyte proliferation and CTL activity were determined according to Section 2. Values are mean ± S.D. of 10 mice.

\* Significantly different from S180 control group at  $p < 0.05$ .

\*\* Significantly different from S180 control group at  $p < 0.001$ .

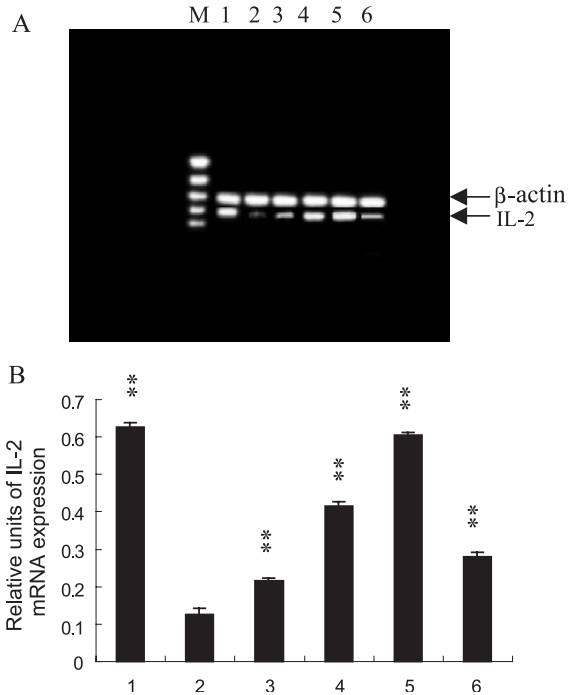


Fig. 1. RT-PCR analysis of IL-2 mRNA in murine spleen (A) PCR products run on an agarose gel. IL-2 (lower panel) and  $\beta$ -actin (upper panel) cDNA fragments were amplified from mice spleen. (B) Comparison of IL-2 mRNA expression level by semiquantitative PCR. The IL-2 mRNA levels were expressed as the ratio of the IL-2 band intensity over the respective  $\beta$ -actin band intensity. Data represent mean ± S.D. of four separated experiments. \*\* ( $P < 0.01$ ) represents significant difference from S180 control. (M) PCR marker (1543, 994, 697, 515, 377 and 237 bp); (1) normal control; (2) S180 control; (3) 20 mg/kg CTX; (4–6) 5, 10 and 20 mg/kg LBP<sub>3p</sub>, respectively.

S180-bearing mice (Table 2). The effect of LBP<sub>3p</sub> 10 mg/kg was the best and it could restore macrophage phagocytosis and humoral immunity in S180-bearing mice closely to those of normal control.

#### 3.3. Effects of LBP<sub>3p</sub> on cellular immunity in S180-bearing mice

Spleen lymphocyte proliferation and CTL activity were significantly decreased in S180-bearing mice. LBP<sub>3p</sub> could remarkably increase spleen lymphocyte proliferation and CTL activity in S180-bearing mice (Table 3). LBP<sub>3p</sub> 10 mg/kg could regulate cellular immunity in S180-bearing mice nearly to that of normal control.

Table 4  
Effects of LBP<sub>3p</sub> on lipid peroxidation in S180-bearing mice

Group	Concentration (mg/kg)	Serum ( $\mu$ mol/l)	Tissue (nmol/g)	
			Liver	Spleen
Normal control	–	3.606 $\pm$ 0.219***	416.34 $\pm$ 17.52***	257.98 $\pm$ 18.61***
S180 control	–	4.302 $\pm$ 0.131	499.00 $\pm$ 14.14	370.66 $\pm$ 25.17
CTX	20	3.981 $\pm$ 0.132***	473.41 $\pm$ 12.29*	344.29 $\pm$ 17.68*
LBP <sub>3p</sub>	5	3.830 $\pm$ 0.117***	452.08 $\pm$ 24.09***	304.60 $\pm$ 20.91**
	10	3.651 $\pm$ 0.138***	425.39 $\pm$ 25.14***	255.25 $\pm$ 17.49**
	20	3.920 $\pm$ 0.147***	463.68 $\pm$ 19.31**	335.07 $\pm$ 19.47*

S180-bearing mice were administered p.o. with CTX 20 mg/kg and LBP<sub>3p</sub> 5, 10, 20 mg/kg for 10 days once daily. Normal control and S180-bearing control groups received the same volume of normal saline. MDA contents in liver, spleen and serum were determined. Values are mean  $\pm$  S.D. of 10 mice.

\*Significantly different from S180 control group at  $p < 0.05$ .

\*\*Significantly different from S180 control group at  $p < 0.01$ .

\*\*\*Significantly different from S180 control group at  $p < 0.001$ .

### 3.4. Effects of LBP<sub>3p</sub> on IL-2 gene expression in S180-bearing mice

Effect of LBP<sub>3p</sub> on IL-2 mRNA level in murine spleen was determined by a semi-quantitative RT-PCR. As seen in Fig. 1, the expression level of IL-2 was significantly decreased in S180-bearing mice compared with that in normal control. LBP<sub>3p</sub> markedly augmented IL-2 gene expression in S180-bearing mice. LBP<sub>3p</sub> 5, 10 and 20 mg/kg increased IL-2 mRNA level by 3.29-, 4.80- and 2.23-fold, respectively.

### 3.5. Effects of LBP<sub>3p</sub> on lipid peroxidation in S180-bearing mice

The effects of LBP<sub>3p</sub> on lipid peroxidation in S180-bearing mice are shown in Table 4. The results showed that MDA contents were increased in liver, spleen and serum of S180-bearing mice and LBP<sub>3p</sub> could significantly inhibit the lipid peroxidation. LBP<sub>3p</sub> 10 mg/kg could decrease the lipid peroxidation in S180-bearing mice nearly to that of normal control.

## 4. Discussion

The relation between the occurrence, growth and decline of tumor and immune states is the essential problem of tumor immunology. The discovery and identification of new antitumor drugs, which can potentiate the immune function has become an important goal of research in immunopharmacology and onco-

therapy. This study demonstrates the favourable anti-tumor effect of LBP<sub>3p</sub>, a polysaccharide–protein complex from *L. barbarum* and its immunomodulative activity. LBP<sub>3p</sub> could significantly inhibit the growth of mouse transplantable sarcoma S180 after treatment for 10 days and the inhibitory effect of LBP<sub>3p</sub> 10 mg/kg was close to that of CTX 20 mg/kg, a positive control.

Protective immunity against tumor is composed of both humoral and cellular immunity. The humoral defence via antibody response is mediated by B cells and other immune cells involved in antigen processing and immunization. The antigen–antibody complex can counteract toxin and defend the infection induced by pathogen. Cell-mediated immune defense was mediated specifically by T cells including cytotoxic T cells. T cells can kill tumors and produce many lymphocyte factors consisting of macrophage mobile factor, lymphotoxin, transfer factor and interferon, which can enhance macrophage phagocytosis and the capacity of killing target cells [16]. In this experiment, we found that LBP<sub>3p</sub> could significantly increase macrophage phagocytosis, the antibody secreted by spleen cells, spleen lymphocyte proliferation, CTL activity in S180-bearing mice that suggested that LBP<sub>3p</sub> could enhance the humoral immunity and cell-mediated immunity in S180-bearing mice. LBP<sub>3p</sub> has potent tumor therapeutic activity by improving the immune system.

Since cytokines play a prominent role in the development of immune response, we also investigated the effect of LBP<sub>3p</sub> on the production of IL-2 in S180-bearing mice. IL-2 stimulates the proliferation of CTL, helper T lymphocytes, natural killer (NK) cells, lym-

phokine activated killer (LAK) cells and macrophages, all of which can participate in immunological antitumor mechanisms [17]. IL-2 mRNA level was found to be augmented in LBP<sub>3p</sub>-treated S180 murine spleen in the present study, which implied that IL-2 may be involved in the early phase of immune response and induces CTL production. The increase in IL-2 also may explain the antitumorigenic properties of LBP<sub>3p</sub>.

The function of immune cells largely relies on the structures and functions of their membranes [18]. Membrane phospholipid is the main object attacked by free radical. The more the degree of unsaturation of membrane phospholipid, the more the lipid peroxidation and the damage to cell membranes. The damages to the structure and function of the membranes of immune cells induced by lipid peroxidation have an adverse effect on the immune function. The present study showed that LBP<sub>3p</sub> significantly decreased the lipid peroxidation of immune cells in S180-bearing mice, demonstrating indirectly that LBP<sub>3p</sub> could potentiate the immune function in S180-bearing mice to some extent.

In this experiment, the inhibitory effects of LBP<sub>3p</sub> on tumor and its immunomodulative activity were not in a dose-dependent manner. The effect of LBP<sub>3p</sub> 10 mg/kg was the best, LBP<sub>3p</sub> 5 mg/kg taking second place. It was reported that the key to regulate the immune function by polysaccharide was the level of state in body, not the dose. The immune level can be regulated to the normal by polysaccharide in autoimmunity process and no effect was evident at higher doses above the limit. It was achieved by integral harmony function in which network of immune-neuroendocrine interactions was the priority [19].

In conclusion, the antitumor and immunostimulatory activities of LBP<sub>3p</sub> have been demonstrated in mice. Further studies on the mechanism by which LBP<sub>3p</sub> induces these effects and additional clinical usefulness in therapies of cancer are needed.

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