Carbohydrate Polymers 90 (2012) 1114–1119

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

A polysaccharide from *Sargassum fusiforme* protects against immunosuppression in cyclophosphamide-treated mice

Xiaoming Chen*[,1], Wenjian Nie[1], Sairong Fan[1], Junfeng Zhang, Yanxing Wang, Jianxin Lu, Liqin Jin*

Institute of Glycobiological Engineering/School of Laboratory Medicine & Life Science, Wenzhou Medical College, Wenzhou 325035, China

ARTICLE INFO

Article history:
Received 27 March 2012
Received in revised form 12 June 2012
Accepted 18 June 2012
Available online 27 June 2012

Keywords:
Sargassum fusiforme
Polysaccharide
Immunomodulatory
Immunosuppession

ABSTRACT

A water-soluble polysaccharide (SFPS) isolated from *Sargassum fusiforme* was purified by DEAE-52 cellulose anion-exchange and Sephadex G-200 gel filtration chromatography. The high performance gel permeation chromatography (HPGPC) analysis showed that the average molecular weight (Mw) of SFPS was 299 kDa. The SFPS was composed of D-fucose, L-xylose, D-mannose and D-galactose in a molar ratio of 5.9:1.0:2.3:2.2. The results showed that SFPS stimulated proliferation and the cytokines (IL-2, IL-6 and IFN-γ) secretion of splenic lymphocytes in cyclophosphamide-induced immunosuppressed mice. SFPS markedly increased the phagocytic rates and cytokines (IL-2, IL-6 and TNF-α) secretion of peritoneal macrophages. Administration of SFPS significantly raised spleen index. It could act as an efficacious adjacent immunopotentiating therapy or an alternative means in lessening chemotherapy-induced immunosuppression, and also can be utilized as immunostimulants for food and pharmaceutical industries.

1. Introduction

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (Jemal et al., 2011). Chemotherapy is one of the most frequently used therapeutic modalities for the treatment of cancer. However, they can also harm healthy cells and lead to multiple organs damage and immunosuppression, which limit their use in tumor treatment (Ehrke, 2003). In recent years, polysaccharides isolated from natural source have been shown to profoundly affect the immune system both in vivo and in vitro with relative nontoxicity and no significant side effects, and therefore are ideal candidates as immunomodulator with a wide application (Chen et al., 2010; Yang, Zhao, & Lv, 2008).

Sargassum fusiforme (Harv.) Setchel, a kind of brown algae widely distributed in China, Korea, and Japan, has been applied as a therapeutic for thousands of years (Zhu et al., 2003). The polysaccharides (SFPS) extracted from *S. fusiforme* in medicine have been demonstrated to have multiple functions, such as antitumor (Yan, Liang, & Li, 2006), anti-HIV (Paskaleva et al., 2006), and antihyperlipidemia (Mao, Li, Gu, Fang, & Xing, 2004). Our previous studies have shown that SFPS could significantly inhibit the growth of A549 lung adenocarcinoma in mice, and had remarkable cytotoxicity to A549 cells (Chen et al., 2012). However, the protective effect of SFPS against immunosuppression is poorly understood, and the basic physicochemical characterization of the polysaccharide is not clear. Thus the present study was designed to investigate the effects of SFPS in immunosuppressed mice induced by cyclophosphamide (CTX) treatment.

In the present study, the polysaccharide fraction from *S. fusiforme* was isolated and purified, and the basic physicochemical characterization was investigated. Then, the immunomodulatory effect of SFPS was elucidated by its effects on splenocyte proliferation, splenocyte and peritoneal macrophage cytokine expression, and macrophage phagocytosis in mice immunosuppressed by treatment with CTX for identifying what extent SFPS is able to restore deviated immune parameters in immunosuppressed animals.

2. Materials and methods

2.1. Materials

S. fusiforme was collected in Dongtou, Zhejiang province (China). Cyclophosphamide (CTX) was provided by the 2nd Affiliated Hospital of Wenzhou Medical College (Zhejiang, China). Sephadex G-200, DEAE-52 cellulose, T-series dextran, dimethyl sulfoxide (DMSO), trifluoroacetic acid (TFA) and standard sugars were purchased from Sigma Chemical Co. Fetal bovine sermon (FBS) and RPMI1640 medium were purchased from Gibco. Cytokine detecting ELISA kits were purchased from Changfeng Biotechnology Co. Ltd. (Zhejiang, China). All the other reagents were analytical grade.

* Corresponding authors. Tel.: +86 577 86699652; fax: +86 577 86689717.
E-mail address: xmchen01@163.com (X. Chen).
[1] These authors contributed equally to this work.

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.06.052

2.2. Animals

ICR mice (8 weeks old, 18–20 g) used for experiments were provided by the Shanghai Laboratory Animal Center (Shanghai, China). The animals were acclimatized for 1 week before being used for the experiment. Before and during the experiment the mice were housed under controlled environmental conditions of temperature (25 ± 2 °C) and a 12 h light and dark cycle, and maintained on (unless otherwise stated) standard food pellets and tap water ad libitum.

All animal (used in this experiment) handling procedures were performed in strict accordance with the P.R. China legislation the use and care of laboratory animals, with the guidelines established by Institute for Experimental Animals of Wenzhou Medical College, and were approved by the college committee for animal experiments.

2.3. Isolation and purification of the polysaccharide

The air dried *S. fusiforme* was soaked with 95% ethanol to remove the pigments and small lipophilic molecules. The residue was then extracted with 10-time volume of distilled water at 90 °C for 3 h thrice. All water-extracts were combined, filtrated, concentrated, and precipitated with 95% ethanol (1: 4, v/v) at 4 °C for overnight. The precipitate was collected by centrifugation and deproteinated by Sevag method (Staub, 1965). Finally the deproteinated supernatant was lyophilized to give crude polysaccharides.

The crude polysaccharides were purified by DEAE-52 cellulose and Sephadex G-200, and the main polysaccharide fraction (SFPS) was collected and lyophilized. SFPS was used for further study.

2.4. Homogeneity and molecular weight

The homogeneity and molecular weight of SFPS was evaluated and determined by high performance gel permeation chromatography (HPGPC). The sample solution was applied to Waters High Performance Liquid Chromatography (HPLC) equipped with a TSK-GEL G5000 PWXL column (7.8 mm × 300 mm), eluted with 0.1 mol/L Na_2SO_4 solution at a flow rate of 0.4 ml/min and detected by a Waters 2414 Refractive Index Detector. The columns were calibrated with Dextran T-series standards (T-500, T-250, T-110, T-70, T-40 and T-10) of known molecular weight. The molecular weight of SFPS was estimated by reference to the calibration curve made above.

2.5. Preliminary characterization of SFPS

The protein content was determined by the method of Bradford (1976) using bovine serum albumin as standard. The content of uronic acid was determined according to the method of Blumenkrantz and Asboe-Hansen (1973) using D-glucuronic acid as the standard. The ester sulfate content of the polysaccharide was evaluated according to method described by Dodgson and Price (1962).

The monosaccharide compositions of SFPS were analyzed by gas chromatography (GC). SFPS was hydrolyzed with 2 M TFA (2 ml) at 120 °C for 2 h. After removing TFA with methanol, the hydrolyzed product was reduced with $NaBH_4$ (50 mg), followed by neutralization with dilute acetic acid and evaporated at 45 °C. The reduced products (alditols) were added with 1 ml pyridine and 1 ml acetic anhydride in a boiling water bath for 1 h. The acetylated derivatives of standard monosaccharides were prepared in the same way. Then, all the derivatives were analyzed by Shimadzu GC-2010 equipped with RTX-50 column (30.0 m × 0.25 mm × 0.25 μm) and flame-ionization detector (FID). The operation was performed using the following conditions: column temperature was programmed from 140 °C (maintained for 2 min) to 170 °C at a rate of 6 °C/min, and increased to 173 °C at a rate of 0.2 °C/min, then increased to 233 °C at a rate of 6 °C/min, held for 40 min at 233 °C; the rate of N_2 carrier gas was 1.0 ml/min; injection temperature was 250 °C; detector temperature was 300 °C.

2.6. UV and infrared spectral analysis

UV absorption spectra was recorder on Thermo Scientific NanoDrop 2000 in the rang of 200–450 cm^{-1}. FT-IR analysis of SFPS was carried out by the potassium bromide (KBr) pellet method with a Bio-Rad FTS 185 spectrometer in the range of 400–4000 cm^{-1}.

2.7. Protocols for immunosuppression induction and treatment

Mice were randomly divided into six groups (six mice in each group). One group of healthy mice was used as normal controls (NS group). From day 1 to day 3, the other five groups of mice were subjected to immunosuppression by administration of CTX (80 mg/kg/d) intraperitoneally. One group of those CTX-treated mice was used as a model group. From day 4 to day 10, the mice were administered the following: NS group, normal saline; model group, normal saline; three SFPS groups, 50, 100 or 200 mg/kg body weight SFPS; and positive control group, 50 mg/kg bodyweight lentinan (LNT). All animals were orally administrated daily.

Twenty-four hours after the last drug administration, mice were weighted and sacrificed by cervical dislocation. The spleen, kidney and liver were immediately removed and weighted. The spleen, kidney or liver index was calculated as spleen, kidney or liver weight/body weight. The spleen sample was used freshly for splenocytes proliferation and cytokines expression assay.

2.8. Splenocyte proliferation assay

Spleen collected from sacrificed mice under aseptic conditions were chopped into small pieces and passed through a fine steel mesh to obtain a homogeneous cell suspension. Recovered spleen cells were resuspended in lysis buffer (0.15 M NH_4Cl, pH 7.4) for 5 min to remove erythrocytes. Then spleen cells were harvested and resuspended in RPMI 1640 complete medium. Cell numbers and viability (over 95%) were assessed microscopically using trypan blue dye exclusion technique. The splenic cells (100 μl) were set up in 96-well plates (1×10^6 cells/well). After the cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 for 48 h, 20 μl MTT (5 mg/ml) were added to each well and incubated for another 4 h. After the incubation, the cell suspensions were centrifuged at 800 rpm for 10 min and the supernatants were removed, then 150 μl of dimethyl sulphoxide (DMSO) working solution was added. The absorbance was measured in an automatic ELISA plate reader at 570 nm after 15 min.

2.9. Phagocytosis of peritoneal macrophages

Peritoneal macrophages were prepared as described (Chen et al., 2008). Phagocytosis of macrophages was measured by neutral red uptake method as described (Weeks, Keisler, Myrvik, & Warinner, 1987). Briefly, macrophages were obtained from the peritoneal exudates. Mice peritoneal exudates cells were harvested by peritoneal lavage using cold phosphate buffered saline (PBS). Cells were then cultured overnight in RPMI-1640 with 10% fetal calf serum (FCS) at 37 °C in a humidified atmosphere of 5% CO_2. The following day, all nonadherent cells were removed by washing with PBS. Adherent cells were detached using 10 mM EDTA in PBS and seeded at a density of 1×10^6 cells/well in the 24-well plate with complete RPMI-1640 media. After 3 h cultured, all nonadherent cells were removed by washing with PBS, and 1 ml 0.1% neutral red

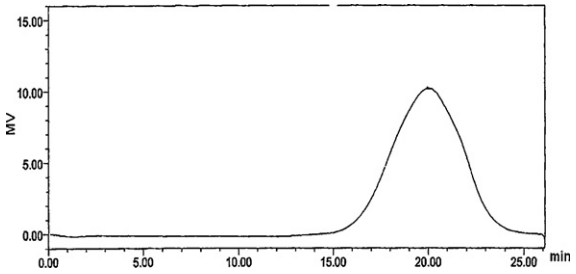

Fig. 1. HPGPC profile of the polysaccharide of SFPS isolated from *S. fusiforme*.

were added and incubated for another 1 h. Then, cells were washed with PBS to remove excess dye. The incorporated dye was resuspended in ethanol (50%) containing glacial acetic acid (1%) and the absorbance was measured at 540 nm in a microplate reader. The absorbance (A) was translated into phagocytic rate for comparison: phagocytic rate = $\text{test}_A/\text{normal control}_A \times 100\%$.

2.10. Determination of cytokine production of peritoneal macrophages (PMφ) and splenic lymphocyte using ELISA

The splenic lymphocyte was prepared as described in Section 2.8, and incubated (1×10^6 cells/well) in 24-well culture plates at 37 °C in humidified 5% CO_2. After 24 h, the supernatants were collected for detection of interleukin-2 (IL-2), interleukin-6 (IL-6) and interferon-γ (IFN-γ) levels using commercial ELISA kits.

After the mice were sacrificed, peritoneal macrophages (PMφ) was prepared as described in Section 2.9, and incubated (1×10^6 cells/well) in 24-well culture plates at 37 °C in humidified 5% CO_2. After 24 h, the supernatants were collected for detection of IL-2, IL-6 and tumor necrosis factor-alpha (TNF-α) levels using commercial ELISA kits.

The assay was carried out according to the procedures recommended in the ELISA kits manual, the absorbance was read at 450 nm on an automatic ELISA plate reader.

2.11. Statistical analysis

All results were presented as mean ± S.D. Data were analyzed by one-way ANOVA using SPSS and Dunnett's test. *P*-values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Isolation and characterization of SFPS

The water-soluble crude polysaccharides were obtained from *S. fusiforme* by hot water extraction, ethanol precipitation, deproteinized, and lyophilized. The extracts were purified through with DEAE-52 cellulose column and Sephadex G-200 column. The main fraction was collected and named as SFPS for further structure characterization and bioactivity assay.

The average molecular weight of SFPS was determined as 299 kDa by HPGPC. The HPGPC profile (Fig. 1) also demonstrated that SFPS had a single and symmetrically peak revealing that SFPS was a homogeneous polysaccharide. The chemical analysis showed that SFPS had a negative response in the Bradford test, indicating the absence of protein. The uronic acid content of SFPS was 6.48%. The ester sulfate content of SFPS was found to be 10.74%. As shown in Fig. 2, the absorption peak near 265 nm detected by the UV spectrum was attributed to —S—O— and $-SO_2-$ structures in the molecule, and indicated the sulfate groups in SFPS. The SFPS composition was found by GC to consist of four kinds of monosaccharide:

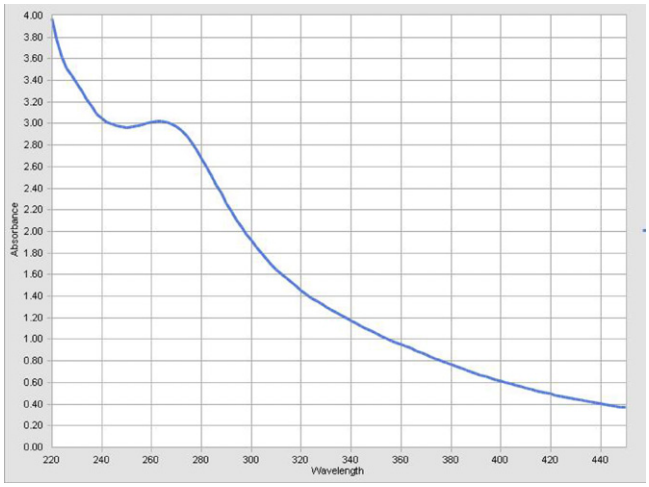

Fig. 2. UV spectrum of the polysaccharide of SFPS isolated from *S. fusiforme*.

D-fucose, L-xylose, D-mannose and D-galactose, whose molar ratios were 5.9:1.0:2.3:2.2, respectively.

As shown in Fig. 3, the IR spectrum of SFPS revealed a typical major broad stretching peak around 3600–3200 cm^{-1} for the hydroxyl group, and the small band at around 2930 cm^{-1} was attributed to the C—H stretching and bending vibrations. The wave number between 1000 and 1200 cm^{-1} is dominated by ring vibrations overlapped with stretching vibrations of (C—OH) side groups and the (C—O—C) glycosidic band vibration (Chen et al., 2011). The relatively strong absorption peak at around 1620 cm^{-1} was attributed to carboxylate O—C—O asymmetric stretching vibrations, and absorption at 1413 cm^{-1} was assigned to C—OH deformation vibration with contribution of O—C—O symmetric stretching vibration of carboxylate group (Fenoradosoa et al., 2010). Absorption peaks at 1257 and 840 cm^{-1} were assigned, respectively, to the stretching vibrations of S=O and C—O—S, an evidence of sulfate ester, confirming directly SFPS was sulfated polysaccharides (Gan, Ma, Jiang, Xu, & Zeng, 2011).

3.2. Effects of SFPS on spleen, liver and kidney index in CTX-treated mice

As shown in Table 1, the spleen index of the animals treated with CTX at dose of 80 mg/kg bodyweight decreased significantly when compared to the normal control which displayed that the immmunosuppressed modeling was built successfully. The liver and kidney indices of animals treated with CTX did not show any difference compared to the normal control. The spleen indices of the animals treated with SFPS of 50,100 or 200 mg/kg increased

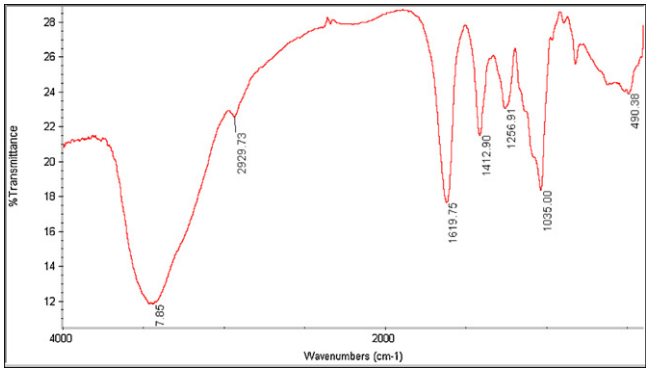

Fig. 3. IR spectrum of the polysaccharide of SFPS isolated from *S. fusiforme*.

Table 1
Effects of SFPS on spleen, liver and kidney index in CTX-treated mice.

Group[a]	Dose (mg/kg)	Spleen index (mg/g)	Liver index (mg/g)	Kidney index (mg/g)
NC	–	4.70 ± 0.64[b]	52.58 ± 3.33	16.73 ± 0.80
MC	–	1.34 ± 0.20	56.34 ± 2.54	16.75 ± 1.21
SFPS	50	7.07 ± 0.82[b]	53.64 ± 2.30	15.06 ± 0.71
	100	7.48 ± 0.64[b]	55.97 ± 5.21	15.56 ± 2.05
	200	6.70 ± 1.56[b]	58.93 ± 7.08	15.28 ± 1.41
LNT	50	7.76 ± 1.30[b]	50.24 ± 5.81	14.35 ± 1.17

[a] NC, normal control; MC, model control; SFPS, *Sargassum fusiforme* polysaccharides; LNT, lentinan (positive control). The values are presented as means ± S.D.
[b] $P < 0.01$ (Compared with model control).

as compared with the model control significantly, although it did not act as a dose-dependent manner. LNT-treated also significantly increased the spleen indices of the animals compared with model control ($P < 0.01$).

3.3. Effect of SFPS on splenocyte proliferation in CTX-treated mice

To understand the mechanism of the immunoregulatory activity of SFPS, its effect on splenocyte proliferation was investigated. As shown in Fig. 4, SFPS significantly increased splenocyte proliferation in a dose-dependent manner at 50, 100 and 200 mg/kg compared to the model group, which suggests that SFPS is directly mitogenic for mouse splenocytes. At the dose of 200 mg/kg, SFPS enhanced splenocyte proliferation about 4-fold. LNT (positive control, 50 mg/kg) also increased splenocyte proliferation significantly when compared to the model control.

3.4. Effects of SFPS on cytokines secretion in the supernatants of splenocytes from CTX-treated mice

To elucidate the immunomodulating effect of SFPS, cytokines (IL-2, IL-6 and IFN-γ) levels in the supernatants of splenocyte cultures from CTX-treated mice were determined by ELISA. As shown in Table 2, the cytokines (IL-2, IL-6 and IFN-γ) secreted by splenocytes were significantly reduced in CTX-treated mice compared with normal control. After SFPS administration, there was a dose-dependent increase of IL-2 production in the supernatants of cultured splenocytes. Similarly, the increase of IL-6 and IFN-γ productions were also observed. The splenocytes from SFPS-treated group (200 mg/kg) secreted more cytokines (IL-2, IL-6 and IFN-γ) productions and the concentrations reached 175.0, 44.2 and 39.01 pg/ml, which were 2.3-, 2.5- and 2.5-fold increases, respectively, compared to the model control group. Significant increases in concentrations of the cytokines (IL-2, IL-6 and IFN-γ) were also

Table 2
Effects of SFPS on cytokines secretion in the supernatants of splenocytes from CTX-treated mice.

Group [a]	Dose (mg/kg)	IL-2 (pg/ml)	IL-6 (pg/ml)	IFN-γ (pg/ml)
NC	–	115.3 ± 7.1[b]	27.6 ± 0.9[b]	25.77 ± 1.43[b]
MC	–	76.0 ± 6.2	17.6 ± 0.4	15.77 ± 1.05
SFPS	50	113.3 ± 4.5[b]	30.3 ± 1.2[b,c]	29.38 ± 2.16[b,c]
	100	149.3 ± 3.2[b,c]	36.4 ± 2.9[b,d]	35.03 ± 2.55[b,d]
	200	175.0 ± 6.2[b,d]	44.2 ± 2.9[b,d]	39.01 ± 3.25[b,d]
LNT	50	134.7 ± 7.4[b,c]	27.7 ± 1.3[b]	29.1 ± 1.91[b,d]

[a] NC, normal control; MC, model control; SFPS, *Sargassum fusiforme* polysaccharides; LNT, lentinan (positive control). The values are presented as means ± S.D.
[b] $P < 0.01$ (compared with model control).
[c] $P < 0.05$ (compared with normal control).
[d] $P < 0.01$ (compared with normal control).

observed in SFPS-treated when compared with normal control ($P < 0.05$ or $P < 0.01$).

3.5. Effect of SFPS on peritoneal macrophages (PMφ) phagocytosis in CTX-treated mice

As a first step towards understanding the effect of SFPS on the activation of peritoneal macrophages, we investigated the effect on the phagocytosis of peritoneal macrophages by neutral red uptake method. As shown in Fig. 5, the phagocytic rate of peritoneal macrophages was significantly reduced in CTX-treated mice compared with normal control. After SFPS treatment, the phagocytic rates of peritoneal macrophages were markedly increased compared with model control. Significant increase in the phagocytic rates was also observed when compared with normal control. Compared to model and normal, SFPS treatment not only restored impaired peritoneal macrophage phagocytosis, but also significantly enhanced the phagocytosis of peritoneal macrophages in a dose-dependent manner.

3.6. Effects of SFPS on cytokines secretion in the peritoneal macrophages (PMφ) from CTX-treated mice

To further elucidate the effect of SFPS on the activation of peritoneal macrophages and the mechanism of the immunoregulatory activity of SFPS, IL-2, IL-6 and TNF-α levels secreted by peritoneal macrophages from CTX-treated mice were determined by ELISA. As shown in Fig. 6, the level of IL-2 and IL-6 in model control was markedly decreased when compared with normal control. The administration of SFPS (50 mg/kg, 100 mg/kg and 200 mg/kg) significantly raised the levels of IL-2, IL-6 and TNF-α

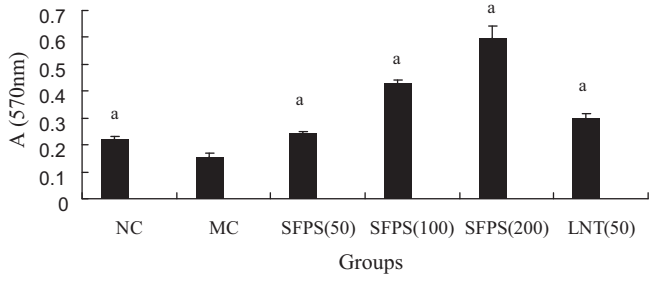

Fig. 4. Effects of SFPS on splenocyte proliferation in CTX-treated mice. NC, normal control; MC, model control; SFPS(50), *Sargassum fusiforme* polysaccharides (50 mg/kg) treated group; SFPS(100), *Sargassum fusiforme* polysaccharides (100 mg/kg) treated group; SFPS(200), *Sargassum fusiforme* polysaccharides (200 mg/kg) treated group; LNT, lentinan (50 mg/kg) treated group (positive control). The values are presented as means ± S.D. [a]$P < 0.01$ (compared with model control).

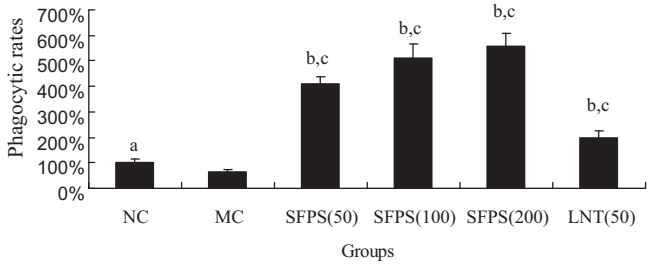

Fig. 5. Effect of SFPS on peritoneal macrophages (PMφ) phagocytosis indicated by Neutral Red assay in CTX-treated mice. NC, normal control; MC, model control; SFPS(50), *Sargassum fusiforme* polysaccharides (50 mg/kg) treated group; SFPS(100), *Sargassum fusiforme* polysaccharides (100 mg/kg) treated group; SFPS(200), *Sargassum fusiforme* polysaccharides (200 mg/kg) treated group; LNT, lentinan (50 mg/kg) treated group (positive control). The values are presented as means ± S.D. [a]$P < 0.01$, [b]$P < 0.001$ (compared with model control); [c]$P < 0.001$ (Compared with normal control).

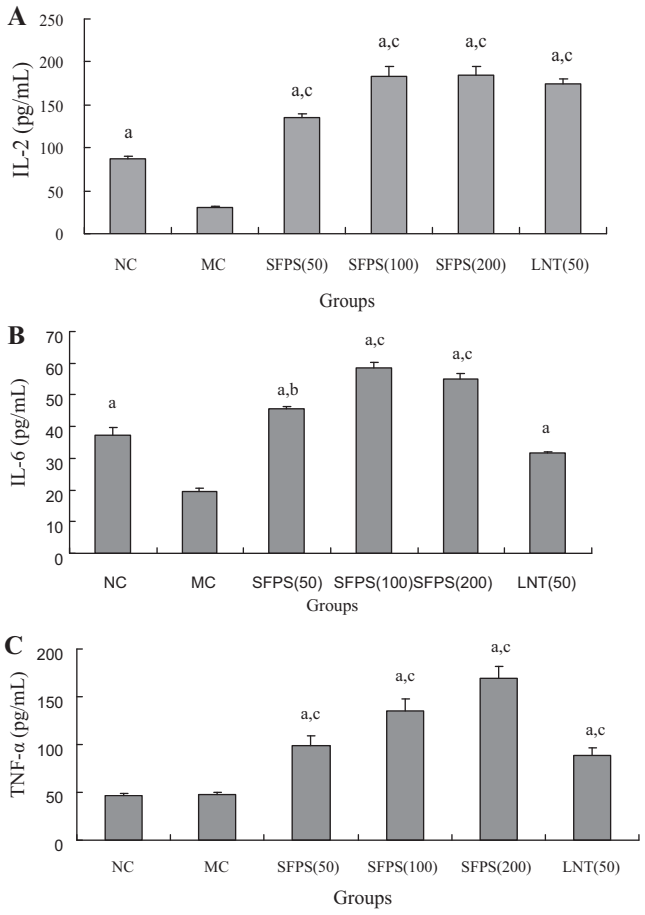

Fig. 6. Effects of SFPS on cytokines secretion in the peritoneal macrophages (PMφ) from CTX-treated mice. (**A**) IL-2 levels in the PMφ from CTX-treated mice; (B) IL-6 levels in the PMφ from CTX-treated mice; (C) TNF-α levels in the PMφ from CTX-treated mice; NC, normal control; MC, model control; SFPS(50), *Sargassum fusiforme* polysaccharides (50 mg/kg) treated group; SFPS(100), *Sargassum fusiforme* polysaccharides (100 mg/kg) treated group; SFPS(200), *Sargassum fusiforme* polysaccharides (200 mg/kg) treated group; LNT, lentinan (50 mg/kg) treated group (positive control). The values are presented as means ± S.D. $^{a}P < 0.01$ (compared with model control); $^{b}P < 0.05$, $^{c}P < 0.01$ (compared with normal control).

when compared with model control ($P < 0.01$), and the levels of TNF-α were increased in a dose-dependent manner. Compared with LNT-treated (50 mg/kg), the peritoneal macrophages from SFPS-treated (50 mg/kg) secreted more IL-6 and TNF-α, except the IL-2.

4. Discussion

Cancer is the subject of widespread fear and taboos. Chemotherapeutics are often used to inhibit the proliferation of caner cells. However, they can also harm healthy cells and cause side effects such as immunosuppression (Wang et al., 2011). Many polysaccharides isolated from plants, fungi, algae and animals are studied in the biomedical area because of their immunostimulating activities (Chen et al., 2010; Schepetkin & Quinn, 2006; Shuai et al., 2010). In the present study, we evaluated the preliminary characterization of SFPS, and investigated the protective effects of SFPS in reversing immunosuppression caused by CTX treatment.

It is well known that CTX is an important chemotherapeutic drug in tumor treatment, but it is adverse to immune system of organism and leads to immunosuppression, which sometimes are life-threatening (Wang et al., 2011). In order to stimulate the state of a weakened immune system in our test model we used mice treated with CTX. The lower spleen index and the decreased capacity of the immune function in the CTX-treated mice in comparison to normal mice indicated an immunosuppressed state which was used for the experiment in our study.

The immune system is the organism's ultimate defense against infectious diseases. The immunologic action of polysaccharides may begin with activating effector cells such as lymphocytes and macrophages et al. Therefore, we investigated the effect of SFPS on peritoneal macrophages and splenocyte. As one of the important immune organs, the spleen index reflects the immune function of the organism. Immunopotentiator could increase the weight of spleen. In this study, the effects of SFPS on spleen index and splenocyte proliferation were measured. The increase in spleen index and splenocyte proliferation of mice treated with SFPS indicated that SFPS was able to against the immunosuppression induced by CTX.

Immunostimulation itself is regarded as one of the important strategies to improve the body's defense mechanism. Many polysaccharides could modulated cytokine and/or chemokine production, and stimulate production of both pro-inflammatory and anti-inflammatory cytokines (Xie et al., 2008). In the innate and adaptive immuneresponses, activated splenic lymphocytes play an important role by producing cytokines, IL-2, IL-6, IFN-γ and other inflammatory mediators. IL-2 is an important immune factor secreted by helper T lymphocytes, which can promote immune cell proliferation and differentiation (Im, Kim, & Lee, 2006). It has many immunopotentiating effects and exhibit high cytotoxic activity against autologous tumor cells (Yuan, Song, Li, Li, & Dai, 2006). Among the pro-inflammatory cytokines, IL-6 is one of the most important immune and inflammatory mediators that regulate diverse cell functions including proliferation and differentiation of B-cells and T-cells (Sobota et al., 2008). IFN-γ possesses a wide spectrum of biological effects and is one of the major immunoregulatory molecules that induce effective immune responses against bacteria and exogenous infectious agents (Blankenstein & Qin, 2003; Sugisaki et al., 2009). The present results showed that SFPS significantly increased cytokines (IL-2, IL-6 and IFN-γ) expression in a dose-dependent manner, and high-dose SFPS-treated (100 mg/kg and 200 mg/kg) could secrete more cytokines (IL-2, IL-6 and IFN-γ) in compared with normal control, suggesting that SFPS not only reversed the splenocytes function reduced by CTX, but also could markedly improve splenocytes function in CTX-inhibited mice.

Phagocytes (neutrophils, monocytes and macrophages) are key participants in the innate immune response, and phagocytic function is commonly used in evaluating the non-specific immune status of animals (Chen et al., 2010). Macrophage is the most important professional phagocyte, and plays an essential and pivotal role in host defense against any type of invading cells including tumor cells (Katsiari, Liossis, & Sfikakis, 2010). The present study showed that SFPS significantly enhanced the phagocytosis of peritoneal macrophages in a dose-dependent manner, suggesting that SFPS could enhance the non-specific immune function in CTX-treated mice.

In the innate and adaptive immuneresponses, activated macrophage play an important role. Macrophages maintain an effective immune response at the site of inflammation and malignancy (Biswas & Sodhi, 2002). Secretion of cytokines by activated macrophages is central to their immunoregulatory role and the orchestration of a robust immune response by macrophages. TNF-α plays a pivotal role in host defense and can induce the expression of a number of other immunoregulatory and inflammatory mediators (Baugh & Bucala, 2001). Ability to enhance production of cytokines, such as interleukins, TNF-α, has been widely used as an ex vivo experimental model for studying immunomodulatory activity of polysaccharides. Therefore, in the experiment, we examined whether SFPS activated peritoneal macrophages to induce effector molecules such as IL-2, IL-6 and TNF-α. The present results

showed that SFPS significantly enhanced the secretion of IL-2, IL-6 and TNF-α of peritoneal macrophages. Thus, the polysaccharide could reverse the immunosuppression induced by CTX and improve the immune function thought activating peritoneal macrophages.

In conclusion, the polysaccharide SFPS was successfully isolated from *S. fusiforme*, and purified by DEAE-52 cellulose and Sephadex G-200. The complete structure of SFPS was not obtained, but some important characterization was established. Furthermore, the present study demonstrates, for the first time, that SFPS can reverse the immunosuppression in CTX-treated mice. Although the exact underlying mechanism of SFPS is unknown, based on the results presented above, we conclude that the chemoprotective activity conferred by SFPS may be attributed to its capacity to enhance spleen index, splenocyte proliferation, phagocytosis of peritoneal macrophages and activated splenocyte and peritoneal macrophage. Above all, our findings may provide a mechanistic basis for using SFPS as an efficacious adjacent immunopotentiating therapy or an alternative means in lessening chemotherapy-induced immunosuppression, and also can be utilized as immunostimulants for food and pharmaceutical industries.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20972116), Natural Science Foundation of Zhejiang Province of China (No. Y507096) and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

References

Baugh, J. A., & Bucala, R. (2001). Mechanisms for modulating TNF-α in immune inflammatory disease. *Current Opinion in Drug Discovery & Development*, *4*, 635–650.

Biswas, S. K., & Sodhi, A. (2002). In vitro activation of murine peritoneal macrophages by monocyte chemoattractant protein-1: Upregulation of CD11b, production of proinflammatory cytokines and the signal transduction pathway. *Journal of Interferon & Cytokine Research*, *5*, 527–538.

Blankenstein, T., & Qin, Z. (2003). The role of IFN-c in tumor transplantation immunity and inhibition of chemical carcinogenesis. *Current Opinion in Immunology*, *15*, 148–154.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry*, *54*, 484–489.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, *72*, 248–254.

Chen, J. R., Yang, Z. Q., Hu, T. J., Yan, Z. T., Niu, T. X., Wang, L., et al. (2010). Immunomodulatory activity in vitro and in vivo of polysaccharide from *Potentilla anserine*. *Fitoterapia*, *81*, 1117–1124.

Chen, X. M., Jin, J., Tang, J., Wang, Z. F., Wang, J. J., Jin, L. Q., et al. (2011). Extraction, purification characterization and hypoglycemic activity of a polysaccharide isolated from the root of *Ophiopogon japonicus*. *Carbohydrate Polymers*, *83*, 749–754.

Chen, X. M., Lu, J. X., Zhang, Y. D., He, J. T., Guo, X. Z., Tian, G. Y., et al. (2008). Studies of macrophage immuno-modulating activity of polysaccharides isolated from *Paecilomyces tenuipes*. *International Journal of Biological Macromolecules*, *43*, 252–256.

Chen, X. M., Nie, W. J., Yu, G. Q., Li, Y. L., Hu, Y. S., Lu, J. X., et al. (2012). Antitumor and immunomodulatory activity of polysaccharides from *Sargassum fusiforme*. *Food and Chemical Toxicology*, *50*, 695–700.

Dodgson, K. S., & Price, R. G. (1962). A note on the determination of the ester sulfate content of sulfated polysaccharides. *Biochemistry Journal*, *84*, 106–110.

Ehrke, M. J. (2003). Immunomodulation in cancer therapeutics. *International Immunopharmacology*, *3*, 1105–1119.

Fenoradosoa, T. A., Ali, G., Delattre, C., Laroche, C., Petit, E., Wadouachi, A., et al. (2010). Extraction and characterization of an alginate from the brown seaweed *Sargassum turbinarioides* Grunow. *Journal of Applied Phycology*, *22*, 131–137.

Gan, D., Ma, L. P., Jiang, C. X., Xu, R. J., & Zeng, X. X. (2011). Production preliminary characterization and antitumor activity in vitro of polysaccharides from the mycelium of *Pholiota dinghuensis* Bi. *Carbohydrate Polymers*, *84*, 997–1003.

Im, S. A., Kim, K., & Lee, C. K. (2006). Immunomodulatory activity of polysaccharides isolated from *Salicornia herbacea*. *International Immunopharmacology*, *6*, 1451–1458.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *A cancer Journal for Clinicians*, *61*, 69–90.

Katsiari, C. G., Liossis, S. C., & Sfikakis, P. P. (2010). The pathophysiologic role of monocytes and macrophages in systemic lupus erythematosus: A reappraisal. *Seminars in Arthritis & Rheumatism*, *39*, 491–503.

Mao, W. J., Li, B. F., Gu, Q. Q., Fang, Y. C., & Xing, H. T. (2004). Preliminary studies on the chemical characterization and antihyperlipidemic activity of polysaccharide from the brown alga *Sargassum fusiforme*. *Hydrobiologia*, *512*, 263–266.

Paskaleva, E. E., Lin, X. D., Li, W., Cotter, R., Klein, M. T., Roberge, E., et al. (2006). Inhibition of highly productive HIV-1 infection in T cells, primary human macrophages, microglia, and astrocytes by *Sargassum fusiforme*. *AIDS Research and Therapy*, *3*, 15.

Schepetkin, I. A., & Quinn, M. T. (2006). Botanical polysaccharides: Macrophage immunomodulation and therapeutic potential. *International Immunopharmacology*, *6*, 317–333.

Shuai, X. H., Hu, T. J., Liu, H. L., Su, Z. J., Zeng, Y., & Li, Y. H. (2010). Immunomodulatory effect of a *Sophora subprosrate* polysaccharide in mice. *International Journal of Biological Macromolecules*, *46*, 79–84.

Sobota, R. M., Müller, P. J., Khouri, C., Ullrich, A., Poli, V., Noguchi, T., et al. (2008). SHPS-1/SIRP1α contributes to interleukin-6 signalling. *Cellular Signalling*, *20*, 1385–1391.

Staub, A. M. (1965). Removal of proteins from polysaccharides. *Methods in Carbohydrate Chemistry*, *5*, 5–7.

Sugisaki, H., Yamanaka, K., Kakeda, M., Kitagawa, H., Tanaka, K., Watanabe, K., et al. (2009). Increased interferon-γ, interleukin-12p40 and IL-8 production in *Propionibacterium acnes*-treated peripheral blood mononuclear cells from patient with acne vulgaris. *Journal of Dermatological Science*, *55*(1), 47–52.

Wang, H., Wang, M. Y., Chen, J., Tang, Y., Dou, J., Yu, J., et al. (2011). A polysaccharide from *Strongylocentrotus nudus* eggs protects against myelosuppression and immunosuppression in cyclophosphamide-treated mice. *International Immunopharmacology*, *11*, 1946–1953.

Weeks, B. A., Keisler, A. S., Myrvik, Q. N., & Warinner, J. E. (1987). Differential uptake of neutral red by macrophages from three species of estuarine fish. *Development and Comparative Immunology*, *11*, 117–124.

Xie, G., Schepetkin, I. A., Siemsen, D. W., Kirpotina, L. N., Wiley, J. A., & Quinn, M. T. (2008). Fractionation and characterization of biologically-active polysaccharides from *Artemisia tripartite*. *Phytochemistry*, *69*, 1359–1371.

Yan, L. L., Liang, Q., & Li, J. C. (2006). Apoptosis in Lovo cells induced by SFPS was associated with a activation of caspase-3 mediated by caspase-9. *Chinese Journal of Cell Biology*, *28*, 193–200.

Yang, X. B., Zhao, Y., & Lv, Y. (2008). In vivo macrophage activation and physicochemical property of the different polysaccharide fractions purified from *Angelica sinensis*. *Carbohydrate Polymers*, *71*, 372–379.

Yuan, H. M., Song, J. M., Li, X. G., Li, N., & Dai, J. C. (2006). Immunomodulation and antitumor activity of j-carrageenan oligosaccharides. *Cancer Letters*, *243*, 228–234.

Zhu, W., Ooi, V. E. C., Chan, P. K. S., & Ang, P. O., Jr. (2003). Isolation and characterization of a sulfated polysaccharide from the brown alga *Sargassum patens* and determination of its anti-herpes activity. *Biochemistry and Cell Biology*, *81*, 25–33.