Differential effect of *Coriolus versicolor* (Yunzhi) extract on cytokine production by murine lymphocytes in vitro[☆]

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Abstract

Being one of the commonly used Chinese medicinal herbs, *Coriolus versicolor* (CV), also named as Yunzhi, was known to possess both anti-tumor and immunopotentiating activities. The present study aimed to investigate the in vitro immunomodulatory effect of a standardized ethanol–water extract prepared from CV on the proliferation of murine splenic lymphocytes using the MTT assay, and the production of six T helper (Th)-related cytokines using the enzyme-linked immunosorbent assay (ELISA) technique. The results showed that the CV extract significantly augmented the proliferation of murine splenic lymphocytes in a time- and dose-dependent manner, maximally by 2.4-fold. Moreover, the production of two Th1-related cytokines, including interleukin (IL)-2 and IL-12, in culture supernatants from the CV extract-activated lymphocytes was prominently upregulated at 48 and 72 h. Positive correlations were found between the levels of these two cytokines and the MTT-based proliferative response. In contrast, the production of two other Th1-related cytokines, including interferon (IFN)-γ and IL-18, was significantly augmented only at 24 h, but not at 48 and 72 h. On the other hand, the levels of two Th2-related cytokines such as IL-4 and IL-6 were undetectable in the culture supernatants of lymphocytes treated with the CV extract. The CV extract was suggested to be a lymphocyte mitogen by differentially enhancing the production of Th1-related cytokines.
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Keywords: Coriolus versicolor; Lymphocytes; Immunomodulatory; T helper cytokines; Interleukin-2; Interleukin-12

1. Introduction

Plant-derived extracts have been historically considered as an important remedy for maintaining health, enhancing overall immune status, and prevention and treatment of chronic diseases [1]. Recent studies have demonstrated that mushroom extracts possess a wide range of biological activities including antiviral, anti-tumor and immunopotentiating activities [2–6]. *Coriolus versicolor* (CV), known as Yunzhi in China, is a mushroom species belonging to the Basidiomycetes class of fungi. The medicinal value of Yunzhi was recorded in the *Compendium of Chinese Materia Medica* and *Shen Non Compendium Medica* thousands of years ago in China. Nowadays, its therapeutic potentials have been gaining acceptance among patients worldwide [3,6].

Among various bioactive components derived from CV, polysaccharopeptide (PSP) was found to be the most prominent in anti-tumor and immunomodulatory efficacy. PSP is a protein-bound polysaccharide isolated from the deep-layer cultivated mycelia of *C. versicolor* COV-1 strain [7]. The polysaccharide moiety of PSP is a β (1→3)-glucan branching at 4′ and 6′ positions, and consists of five different sugars including arabinose, glucose, galactose, mannose and xylose. Its polysaccharide moiety is rich in aspartic and glutamic acids. The substance has a molecular weight of about 100,000 and is highly water-soluble [4,8]. Previous in vitro studies have reported that ethanol–water extract of CV [9], PSP and PSK (protein-bound polysaccharide Krestin) [10] can directly inhibit the proliferation of leukemia, lymphoma, hepatoma, breast and lung tumor cell lines in a dose-dependent manner [3,11–16]. PSP was also found to stimulate both humoral and cell-mediated immune responses. It can induce the production of interferon (IFN)-γ and interleukin (IL)-2, increase the T-cell proliferation and counteract the immunosuppression induced by cytotoxic drugs such as cyclophosphamide [8,16–18]. Another research group reported that the water extract of CV (I'm-Yunity™) significantly increased IL-1β and IL-6 secretion while substantially lowering IL-8 production of HL-60 leukemic cells in vitro, suggesting the extract may play key roles in the mediation of inflammatory and immune responses [14]. In clinical trials, PSP administration to patients with esophageal cancer, gastric cancer and lung cancer, who are undergoing radiotherapy or chemotherapy, can help to alleviate symptoms and prevent the decline in immune status [3,4,19]. A recent study has demonstrated that regular consumption of capsules containing both Yunzhi and Danshen (*Salvia miltiorrhiza*) extract could improve cellular immunity in normal healthy subjects by elevating the gene expression of IL-2 receptor in peripheral blood mononuclear cells, and increasing the percentage and absolute counts of T helper (Th) cells [20]. It has been demonstrated that Th1/Th2 balance plays important roles in anti-tumor immunity in which Th1 cells producing IFN-γ and IL-2 are essential for inducing cellular and tumor immunity, whereas Th2 cells producing IL-4 and IL-6 are associated with suppression of cytolytic activity [21,22].

The objective of the present study was to examine the in vitro immunomodulatory effects of a wildly grown *C. versicolor* ethanol–water standardized extract, supplied by Vita Green Health Products, on murine splenic lymphocytes. The test parameters included the proliferation response of murine lymphocytes using MTT proliferation assay, and the production of six Th1- and Th2-related cytokines using ELISA technique.

2. Materials and methods

2.1. Preparation of CV extract

An ethanol–water extract from *C. versicolor* L. of Polyporaceae family was provided by Vita Green Health Products, Hong Kong, in compliance with Good Manufacturing Practice (GMP) standard. The extract was obtained from the fruiting bodies of wildly grown *C. versicolor* in the Guangxi province of mainland China. The *C. versicolor* was authenticated by Prof. Zhang Xiao-qing (Mycology expert, Institute of Microbiology Chinese Academy of Sciences, Beijing, China). Herbarium voucher specimen (no. 2003-2510) is deposited at the museum of the Institute of Chinese Medicine, The Chinese University of Hong Kong. The fruiting bodies were cleaned, crushed and extracted with ethanol–water at proprietary proportions and temperatures, followed by differential precipitation with ethanol. Further processing of specific ethanol fractions led to isolation of the extraction product in

powdered form (Batch no.: Yz03; shelf time: 3 years). The major components of the resulting CV extract were polysaccharides and triterpenoids, which were approximately 30% by weight. Plain RPMI medium 1640 (Invitrogen GIBCO, New York, USA) was used to dissolve the CV extract as stock solutions of 3 mg/ml for 48 h at room temperature with continuous shaking. Insoluble material was removed by centrifugation and the supernatant was sterilized using a 0.22-μm filter, and further diluted with the plain culture medium to the defined concentrations as indicated.

2.2. Endotoxin assay

Gram-negative bacterial endotoxin level of the CV extract was measured by the Pyrogent 03 plus *Limulus amebocyte* lysate multi-test assay (Bio-Whittaker, Walkersville, MD, USA).

2.3. Preparation of lymphocytes from mouse spleens

Inbred BALB/c mice, 6–8 weeks of age, were fed and kept in the University Laboratory Animal Services Centre, The Chinese University of Hong Kong. Mice were sacrificed by cervical dislocation and the spleens were removed aseptically. The spleens were cut into several pieces and pressed through a stainless steel mesh using a syringe plunger. The spleen cell suspension was washed twice with phosphate buffered saline (PBS, Invitrogen GIBCO), and centrifuged twice at $400 \times g$ for 5 min. The resulting cell pellet was resuspended in RPMI medium 1640 supplemented with 10% fetal bovine serum (FBS, Invitrogen GIBCO). The murine splenocytes were then slowly layered onto an equal volume of Ficoll-Paque™ PLUS gradient solution (Amersham Pharmacia Biotech, Uppsala, Sweden) in a 15-ml centrifuge tube. After centrifugation at $800 \times g$ for 20 min, lymphocytes were collected at the interface between the two layers. The cells were then washed twice with RPMI medium, and resuspended in RPMI medium supplemented with 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin (Invitrogen GIBCO) for subsequent experiments.

2.4. Cell cultures

Murine splenic lymphocytes were counted using a hemocytometer and cell viability was determined by trypan blue exclusion test. Cells were adjusted to a concentration of 3×10^6 cells/ml and incubated with or without the CV extract (12.5–400 μg/ml) in a total volume of 200 μl of RPMI medium supplemented with 10% FBS per well of a 96-well round-bottomed Costar culture plate (Corning, Acton, MA, USA). Concanavalin A (Con A, Sigma, St. Louis, MO, USA) at its optimal concentration (1 μg/ml) was added as a positive control. After incubation at 37 °C in a 5% CO_2 atmosphere for 24, 48 and 72 h, the culture plate was centrifuged at $800 \times g$ for 3 min and culture supernatants were collected, filtered, and stored at -80 °C for measurements of cytokine production. Proliferative response of the cells remained in the plate was determined by MTT assay.

2.5. MTT proliferation assay

In order to determine the mitogenic (growth-promoting) effect of the CV extract on murine splenic lymphocytes, MTT assay was used to detect the reduction of MTT (3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide, Sigma) by mitochondrial dehydrogenase to blue formazan product, which reflects the normal functioning of mitochondria and hence the metabolic rate of cells. Following incubation of murine splenic lymphocytes with the CV extract for 24, 48 and 72 h, and collection of culture supernatant as previously mentioned, 200 μl of fresh RPMI culture medium was added per well to resuspend the cells. Thirty microliters of 5 mg/ml MTT in PBS was added to each well and the plate was incubated at 37 °C for 2 h. The plate was then centrifuged and followed by removal of medium. One hundred microliters of dimethylsulfoxide (DMSO, Sigma) was then added. After incubation at 37 °C for 5 min, absorbance in control and CV extract-treated cells was measured spectrophotometrically at 540 nm using a Benchmark microtiter plate reader (Bio-Rad Laboratories, Hercules, CA, USA).

2.6. Cytokine production of murine splenic lymphocytes after stimulation

Production of murine IL-2, IL-4, IL-6, IL-12, IL-18 and IFN-γ was measured by enzyme-linked immunosorbent assay (ELISA) according to the instructions of the manufacturer (BD Pharmingen,

California, USA). Briefly, a 96-well flat-bottomed microtiter plate was pre-coated overnight with an anti-mouse cytokine monoclonal antibody (capture antibody). Followed by blocking and several washings, working standards and samples (collected supernatants from CV extract-lymphocyte cultures) were then added for incubation for 2 h. After washings, the working detector solution containing biotinylated anti-mouse cytokine monoclonal antibody and avidin-horseradish peroxidase conjugate was added to each well and incubated for 1 h. Substrate solution was then added for incubation, followed by the addition of stop solution, and the absorbance was read within 30 min using a Benchmark microtiter plate reader (Bio-Rad Laboratories) at 450 nm with wavelength correction at 570 nm.

2.7. Statistical analysis

Results were expressed as the mean±standard error. Pearson's correlation test was used to assess the correlations between variables. Statistical differences were assessed by the Student's unpaired t-test, with $p < 0.05$ as statistically significant.

3. Results

3.1. Endotoxin level of the CV extract

Undetectable endotoxin level was found in the CV extract at 800 μg/ml. Since the detection limit is 0.03 EU/ml, the endotoxin concentration of the extract with negative results of gel clot formation was less than 0.03 EU/ml.

3.2. Mitogenic activity of the CV extract on murine splenic lymphocytes

The CV extract at 12.5 to 400 μg/ml was found to significantly stimulate the proliferation of murine splenic lymphocytes in a dose- and time-dependent manner, using the MTT assay (Fig. 1A). Maximal growth was found after 72 h incubation with 400 μg/ml of the extract, achieving a 2.4±0.2-fold increase when compared with the untreated cells. Significant enhancement in lymphocyte proliferation was only
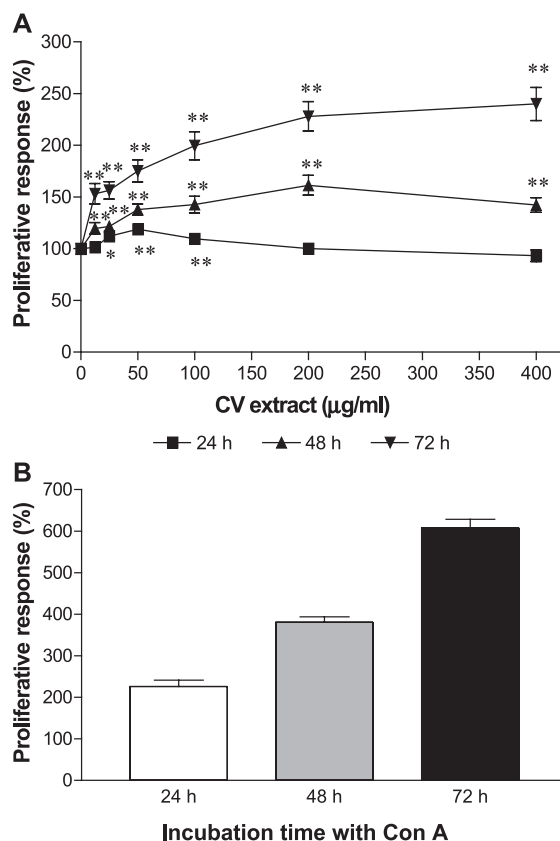

Fig. 1. Mitogenic activity of (A) CV extract and (B) Con A on murine splenic lymphocytes using the MTT assay. Murine splenic lymphocytes were isolated and incubated with increasing concentrations (12.5–400 μg/ml with 2-fold increase) of the CV extract or Con A (1 μg/ml, as positive control) in culture medium for 24, 48 and 72 h. Proliferative response was assessed by the MTT assay for 24 to 72 h, and expressed as the mean % ratio of MTT absorbance in CV extract-treated and control wells±standard error of three independent experiments with six wells each. Differences between the extract-treated and untreated control wells were determined by Student's unpaired t-test. $*p < 0.05$, $**p < 0.01$.

observed after 48 and 72 h of incubation, but not at 24 h. Con A, the positive control, at its optimal concentration (1 μg/ml) was found to prominently promote the lymphocyte growth in a time-dependent fashion, with maximal 6-fold increase at 72 h (Fig. 1B). These results confirmed the mitogenic activity of CV extract on murine splenic lymphocytes. In addition, the extent of growth stimulation by the CV extract at 400 μg/ml after 72 h was similar to that of Con A after 24 h. In order to confirm the mitogenic activity of the CV extract on murine lymphocytes, thymidine

incorporation assay was employed. The CV extract was found to significantly and dose-dependently augment the thymidine incorporation of murine splenic lymphocytes after 72 h, with maximal 3.6-fold

of increase (data not shown). A positive correlation between MTT proliferative response and thymidine incorporation of the CV extract-stimulated lymphocytes was obtained (Pearson r=0.964, $p<0.001$).
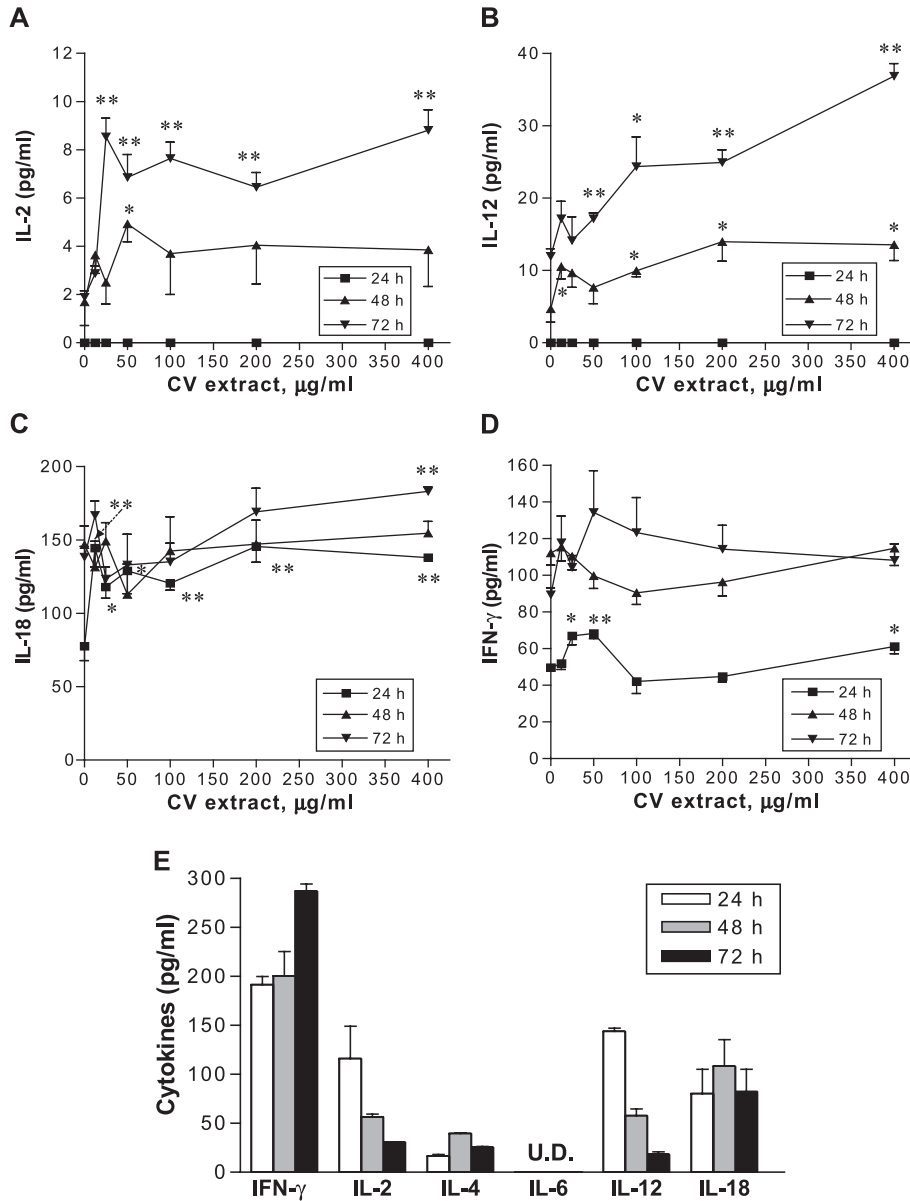

Fig. 2. Productions of (A) IL-2, (B) IL-12, (C) IL-18 and (D) IFN-γ in culture supernatants collected from CV extract-lymphocyte cultures. Murine splenic lymphocytes were isolated and incubated with increasing concentrations (12.5–400 μg/ml with 2-fold increase) of the CV extract in culture medium. Culture supernatants were collected at 24, 48 and 72 h, and the cytokine concentrations were specifically determined by ELISA. (E) Assays of the six cytokines in culture supernatants collected from Con A-lymphocyte cultures at the three time points served as positive controls. Results were expressed as mean concentrations±standard error of quadruplicates. Differences between the extract-treated and untreated control wells were determined by Student's unpaired t-test. *$p<0.05$, **$p<0.01$. U.D.=undetectable.

3.3. Effect of the CV extract on production of Th1- and Th2-related cytokines

Regarding the production of Th1-related cytokines, only very low levels of IL-2 were found in murine splenic lymphocyte culture supernatants after 24 and 48 h exposure to the CV extract. However, after 72 h stimulation, IL-2 production was significantly increased by the CV extract from 25 to 200 µg/ml (Fig. 2A), and was maximal at 400 µg/ml (8.8 ± 0.9 pg/ml, Table 1). Time-dependent secretion of IL-2 was seen at 48 and 72 h, and reached a plateau when starting from 50 µg/ml of the CV extract. IL-12 production was significantly augmented upon incubation with the CV extract in a dose- and time-dependent manner (Fig. 2B), reaching a maximal level at 400 µg/ml after 72 h incubation (36.9 ± 1.7 pg/ml, Table 1). A plateau was reached at 200 µg/ml of the CV extract at 48 h, while the IL-12 production was still highly upregulated at 400 µg/ml of the CV extract at 72 h. Interestingly, IL-18 production was significantly promoted only after 24 h incubation with the CV extract (12.5–400 µg/ml), but not at 48 and 72 h (except at 400 µg/ml, Fig. 2C and Table 1). For IFN-

γ, the CV extract at 25, 50 and 400 pg/ml was found to increase the secretion only at 24 h when compared with the untreated control (Fig. 2D and Table 1). No significant increase in IFN-γ production was found at 48 and 72 h.

Comparing the production of the six cytokines, the CV extract did not induce detectable levels of Th2-related cytokines IL-4 and IL-6 in the splenic lymphocytes culture supernatants (Table 1). Con A, a T-cell mitogen, was found to greatly enhance the

Table 1
Maximal concentrations of six cytokines in culture supernatants of murine splenic lymphocytes incubated with or without the CV extract at three time points

	24 h	48 h	72 h
Cytokine concentration (pg/ml)			
Th1-related cytokine			
IL-2	0.0 ± 0.0	4.9 ± 0.7*	8.8 ± 0.9**
	(0.0 ± 0.0)	(1.7 ± 1.0)	(1.9 ± 0.3)
IL-12	0.0 ± 0.0	14.0 ± 2.7*	36.9 ± 1.7**
	(0.0 ± 0.0)	(4.7 ± 1.8)	(12.0 ± 1.0)
IL-18	145.6 ± 10.7**	154.6 ± 8.1	183.2 ± 3.0**
	(77.5 ± 9.7)	(146.7 ± 12.9)	(138.4 ± 7.2)
IFN-γ	68.2 ± 2.8**	115.2 ± 7.5	134.3 ± 22.8
	(49.6 ± 1.3)	(112.1 ± 6.6)	(89.4 ± 3.7)
Th2-related cytokine			
IL-4	U.D.	U.D.	U.D.
IL-6	U.D.	U.D.	U.D.

Results were expressed as mean concentrations±standard error of quadruplicates. Values in brackets represent the cytokine concentrations in culture supernatants of murine splenic lymphocytes incubated with culture medium alone.
U.D.=undetectable.
* $p<0.05$.
** $p<0.01$.

Table 2
Correlations between the MTT-based proliferative response and cytokine concentrations in culture supernatants of murine splenic lymphocytes incubated with the CV extract

	IL-2	IL-12	IL-18	IFN-γ
Proliferative response (% MTT)				
48 h	0.761	0.789	0.001	−0.631
	(0.047)*	(0.035)*	(0.983)	(0.128)
72 h	0.725	0.895	0.578	0.441
	(0.047)*	(0.007)**	(0.174)	(0.323)

Results were expressed as the Pearson's correlation coefficient r and the p value shown in the bracket using Pearson's correlation test.
* $p<0.05$.
** $p<0.01$.

Table 3
Correlations among the cytokine concentrations in culture supernatants of murine splenic lymphocytes incubated with the CV extract

	IL-2	IL-12	IL-18	IFN-γ
Cytokine concentration (48 h)				
IL-2	–	0.867	0.165	−0.294
		(0.012)*	(0.724)	(0.522)
IL-12	0.867	–	0.466	−0.060
	(0.012)*		(0.292)	(0.893)
IL-18	0.165	0.466	–	0.192
	(0.724)	(0.292)		(0.680)
IFN-γ	−0.294	−0.060	0.192	–
	(0.522)	(0.893)	(0.680)	
Cytokine concentration (72 h)				
IL-2	–	0.607	0.407	0.666
		(0.148)	(0.364)	(0.102)
IL-12	0.607	–	0.336	0.224
	(0.148)		(0.462)	(0.629)
IL-18	0.407	0.336	–	−0.307
	(0.364)	(0.462)		(0.502)
IFN-γ	0.666	0.224	−0.307	–
	(0.102)	(0.629)	(0.502)	

Results were expressed as the Pearson's correlation coefficient r and the p value shown in the bracket using Pearson's correlation test.
* $p<0.05$.

production of four Th1-related cytokines, especially IL-2 and IL-12 to a greater extent at 24 h, when compared with the undetectable levels for the CV extract (Fig. 2E). A remarkable decline was found in the IL-2 and IL-12 secretions after 48 and 72 h, unlike the time-dependent increase for the CV extract. On the other hand, IL-4, a Th2-related cytokine, was induced by Con A and peaked at 48 h, but IL-6 was not detected (Fig. 2E).

Table 2 illustrated that the MTT-based proliferative response of CV extract-induced lymphocytes significantly and positively correlated with IL-2 and IL-12 productions in the culture supernatants after 48 h (IL-2, Pearson $r=0.761$, $p=0.047$; IL-12, Pearson $r=0.789$, $p=0.035$) and 72 h (IL-2, Pearson $r=0.725$, $p=0.047$; IL-12, Pearson $r=0.895$, $p=0.007$). In addition, there was a positive correlation between the concentrations of IL-2 and IL-12 upon stimulation with the CV extract for 48 h (Pearson $r=0.867$, $p=0.012$, Table 3). However, no other significant correlation was found among the other four cytokines (Table 3).

4. Discussion

In this study, the standardized ethanol–water extract of *C. versicolor* in its natural form was shown to significantly and dose-dependently stimulate the proliferation of murine splenic lymphocytes in vitro. Since very low endotoxin level (below 0.03 EU/ml) was found in the CV extract at 800 μg/ml, the observed mitogenic effect of the extract on murine lymphocytes was unlikely to be mediated by the endotoxin (lipopolysaccharide, LPS), which is a strong B-cell mitogen. The proliferative response of splenic lymphocytes in terms of metabolic activity augmented by the extract was assessed by a colorimetric MTT assay (Fig. 1A), and further confirmed by the concomitant increase in DNA synthesis using thymidine incorporation assay, as well as the positive correlation between the two parameters. Con A, a well-known T-cell mitogen used as the positive control, markedly stimulated the proliferation of murine splenic lymphocytes in a time-dependent fashion (Fig. 1B). The present results showed that mitogenic activity of the CV extract was less than that of Con A; however, it could induce comparable mitogenic effect on murine splenic lymphocytes albeit

at higher concentration (400 μg/ml) and longer incubation time (72 h) when compared with Con A (1 μg/ml, 24 h). These results suggested that our CV extract might act as a mitogen for murine splenic lymphocytes since it was reported that PSP promoted the proliferation of T lymphocytes in human peripheral blood and mouse splenocytes [8,16,20]. Our previous in vitro results demonstrated that the same CV extract significantly inhibited the proliferation of human lymphoma (Raji) and leukemic (HL-60, NB-4) cells in a dose-dependent manner, suggesting that the extract differentially modulated the proliferation of normal lymphocytes and leukemia cells [9].

Since cytokines play an important role in regulating the proliferation and differentiation of lymphocytes, the modulatory effects of the CV extract on the production of four Th1-related cytokines (IL-2, IL-12, IL-18 and IFN-γ) and two Th2-related cytokines (IL-4 and IL-6) by the activated lymphocytes were examined using ELISA. It is surprising that although the secretion of IL-2 by murine splenic lymphocytes was significantly augmented after 72 h incubation with the CV extract (Fig. 2A), the increase in IL-2 level occurred much earlier (24 h) when the lymphocytes were treated with Con A, followed by a sharp reduction after 48 and 72 h (Fig. 2E). The IL-2 concentration induced by CV extract was much less than that of Con A, suggesting that the level and time-dependent fashion of IL-2 production from CV extract-induced lymphocytes were totally different from that of Con A. Meanwhile, IL-12 production was found to dose- and time-dependently elevated after 48 and 72 h of incubation with the CV extract (Fig. 2B). Similar to IL-2, the IL-12 production strongly correlated with the MTT-based proliferative response of murine splenic lymphocytes (Table 2), reflecting that both IL-2 and IL-12 might, partly or indirectly, be responsible for promoting the proliferation of murine splenic T lymphocytes. Our results are in concordance with a previous report showing that the immunostimulating activity of PSP and PSK is characterized by their ability to increase white blood cell counts and IL-2 production [8,16,20].

Being not reported in previous studies, our results illustrated that IL-18 production was significantly upregulated upon incubation with the CV extract only at 24 h, but not at 48 and 72 h (Fig. 2C). IL-18 production induced by the CV extract after 48 and 72 h

incubation did not significantly differ from the spontaneous production of IL-18 by untreated splenic lymphocytes. Such early IL-18 induction by the CV extract might be partly related to the activation of NK and CD8$^+$ T cells in the early phase of immune responses since previous studies demonstrated that increased IL-18 secretion might potentiate the cytotoxic actions of NK and CD8$^+$ T cells in response to viral infection and tumor invasion [23,24]. A concomitant increase in IFN-γ production only after 24 h incubation with the CV extract was also found (Fig. 2D), which might be related to the IFN-γ-inducing activity of IL-18. Meanwhile, the pattern of IL-18 and IFN-γ production induced by Con A differed from that of the CV extract after 48 and 72 h of incubation (Fig. 2E), implying that the CV extract differentially enhanced the production of Th1-related cytokines, including IL-2, IL-12, IL-18 and IFN-γ, when compared with Con A. In addition, a water-soluble extract of mushroom *Lentinus lepideus* was shown to activate the expression of transcription factor NF-κB in a human promonocytic cell line U937 after 16 to 20 h of incubation in vitro [25]. It is known that cellular transcription factors (e.g., NF-κB and AP-1) play key roles in regulating cytokine gene expression since their binding sites are mostly found in the promoter sequences of cytokine genes [26,27]. It is possible that early induction of IL-18 and IFN-γ by the CV extract might be related to the transcription factors with same kinetics of induction, like NF-κB, which in turn regulated the expression of mitogenic cytokines IL-2 and IL-12 and hence the lymphocyte proliferation.

No detectable levels of the Th2-related cytokines IL-4 and IL-6 were obtained from the CV extract-activated lymphocytes, suggesting that there might be a Th1-polarized immune response by upregulating the production of Th1-related cytokines. Several studies have shown that Th2 cytokine production was increased in the peripheral blood of tumor-bearing mice or cancer patients [28–30]. In particular, large changes in cytokine environments and subsequent immune dysfunction have been demonstrated in advanced malignant disease [30]. It has been reported that Th1 cytokines enhance the therapeutic efficacy of anti-tumor responses and that Th1-dominant immunity is superior to Th2-dominant immunity in the induction of anti-tumor immunity [21,22,31]. The present results illustrated that the CV extract preferentially augmented the production of Th1-related cytokines from activated splenic lymphocytes, suggesting that it might aid in establishing Th1 dominance which induces cellular immunity in the lymphocyte population that was once Th2-biased due to presence of carcinoma. Further in vitro and in vivo studies are awaited for delineating the relationship between the immunomodulatory effect and anti-tumor actions of the CV extract.

Acknowledgements

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