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Immune enhancement of a polysaccharides peptides isolated from *Coriolus versicolor*

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ABSTRACT A protein–bound polysaccharides (PSP) isolated from *Coriolus versicolor* in Shanghai, at the concentrations of 100–800 μg / ml promoted lymphocyte proliferation. PSP 25 mg / kg *ip* into mice for 5 d antagonized the inhibition of IL–2 production by cyclophosphamide from activated T lymphocytes and restored the suppressed T–cell–mediated delayed type hypersensitivity response to normal. PSP 10–1000 μg / ml induced interferon α and γ production from human peripheral leukocytes 4 and 8 times respectively higher than that of the control groups. Moreover, PSP also increased phagocytic functions of host reticulo–endothelial system. The results suggest that the anti–tumor effects of PSP may be related to its potentiation of host immunological responses.

KEY WORDS *Coriolus versicolor*; glycopeptides; T lymphocytes; interleukin 2 ; cyclophosphamide; interferon type I ; interferon type II ; delayed hypersensitivity

PSP, the protein–bound polysaccharides, extracted from a strain of *Coriolus versicolor* in Shanghai Teachers University, has been proved to be effective against tumor both experimentally and clinically[1,2]. When PSP was combined with chemotherapy, radio–therapy or surgery for cancer patients, it lessened the side effects of radiation, relieved pain and improved appetite, thus improved the whole condition of patients. Its antitumor effects were related to the potentiation of immunological responses, especially T cell mediated immune responses of tumor–bearing hosts[3]. Since T lymphocytes play an important role in immune responses and T cell deficiency existed in many diseases, it is of great interest to investigate if PSP can potentiate T cell functions, such as T lymphocyte proliferation, interleukin–2 (IL–2) production, delayed type hypersensitivity and interferon inductions.

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MATERIALS

PSP: Lot no. K–881224, *m* = 150 kDa, brown powder, soluble in water, was provided by the Mushroom Research Laboratory of Shanghai Teachers University.

Mice Inbred strain C57BL and ICR ♀ mice, 3 months of age, body weight 20 ± SD 2 g ; BALB / cA inbred ♀ mice, 6–8 wk of age, were obtained from Shanghai Animal Center, Chinese Academy of Sciences.

Reagents Concanavalin A (Con A) was purchased from Sigma Co., Cyclophos–phamide (Cyc) from Shanghai No. 12 Pharmaceutical Co., α–methyl–mannoside from Fluka AG and [^{3}H] thymidine from Shanghai Institute of Nuclear Sciences.

Medium RPMI 1640 was purchased from Gibco Laboratories. All RPMI 1640 containing media were supplemented with HEPES buffer 10 mmol / L, penicillin 100 IU / ml, streptomycin 100μg / ml, *L*–glutamine 2 mmol / L, 2–mercaptoethanol (50 μmol / L), and 10% newborn bovine serum (NBS), pH was adjusted to 7.2.

METHODS AND RESULTS

1 Effect on lymphocyte proliferation C57BL ♀ mice were killed by cervical dislocation. The spleens were removed and single cell suspensions were prepared at concentration of 4 × 10^6 cells / ml. For *in vitro* experiments, cells (100 μl) were dispensed into

individual wells of 96 well flat bottom plates (Costar) in the presence of mitogen Con A (5 μg / ml) and various concentrations of PSP (100 μl). Lymphocyte proliferation was expressed by the incorporated radioactivity of $[^3H]$TdR which was added to each well 6 h before harvesting[4]. All experiments were performed at least twice (Tab 1). PSP 100 – 800 μg / ml caused a promoting action on lymphocyte proliferation by increasing DNA synthesis. When T cell mitogen Con A was added, the proliferation of T cells appeared to be more evident. The incorporated dpm value at 800 μg / ml was 4 times higher than that of the control. The T cell–promoting effect of PSP was dose–dependent both in the presence and in the absence of Con A, indicating that PSP *per se* is a T cell stimulator and PSP has also a synergetic action with Con A.

Tab 1. Effect of PSP on T lymphocyte proliferation *in vitro*. The final concentration of mitogen Con A was 5 μg / ml. $n=3$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* control.

PSP (μg / ml)	$[^3H]$TdR incorporation (dpm) without Con A	with Con A
0	1957 ± 378	3333 ± 767
25	1798 ± 383*	2680 ± 480*
50	2535 ± 282*	3750 ± 822*
100	3652 ± 257**	5725 ± 515**
200	3470 ± 720**	8525 ± 2188**
400	3692 ± 782**	14977 ± 2718***
800	5228 ± 1157***	15340 ± 2917***

2 Effect of PSP on IL–2 production in mice Spleen cell suspensions (2 × 10^6 / ml) were prepared. Cell suspensions 2 ml were seeded into wells of 24–well culture plates (Costar) and was incubated at 37℃ in 5% CO_2 incubator for about 24 h in the presence or absence of Con A 5 μg / ml for induction of IL–2. After incubation, the putative IL–2–containing supernatants were harvested and centrifuged to remove cell debris and stored at –25℃. IL–2 activity was determined by activated splenocytes[5]. IL–2 activity was expressed by the ability of IL–2–containing supernatants to support lymphoblast (Con A–activated lymphocytes) growth which was measured by $[^3H]$TdR incorporation into DNA using scintillation counter[5]. The results were listed in Tab 2. PSP 25 mg / kg ip into mice, caused a tendency to increase IL–2 production from splenocytes. Cyclophosphamide (Cyc) 25 mg / kg ip q2d. inhibited IL–2 production ($P<0.01$), but when PSP ip injected daily together with Cyc, the inhibition of IL–2 production by Cyc did not appear any more and IL–2 production reached nearly to that of the normal mice.

Tab 2. Effect of PSP on interleukin 2 production from mouse splenocytes *in vivo*. $n=7$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{***}P<0.01$ *vs* control. $^{++}P<0.01$ *vs* Cyc group.

Treatment	Dose (mg / kg)	IL–2 activity ($[^3H]$TdR dpm)
Control	—	4228 ± 110
PSP	25 × 5 d	5048 ± 257*
Cyc	25 q 2 d.	2595 ± 717**
PSP + Cyc		3957 ± 200++

3 Effect of PSP on interferon (IFN) induction Anticoagulated normal human peripheral leukocytes were separated aseptically from healthy blood donors and made to concentration of 1 × 10^7 cell / ml as IFN inducing cells. For α–IFN induction, Newcastle disease virus (NDV–F line) 64–128 HAU / ml was added into the inducing cells. After spinningly cultured for 24 h at 37℃, the supernatants were harvested, virus was inactivated and preserved for IFN assay. For γ–IFN induction, PHA (100 μg / ml) was used as inducing agent. By means of spinning culture for 48 h at 37℃, the supernatants were harvested and preserved for IFN assay. Human amnionic WISH cells were used as surveying cells. After they formed a monolayer on a 96–well plate in 5% CO_2 incubator at 37℃, serial dilutions of the above induced crude IFN preparations were

added to each well as protectors. Then attacking virus (VSV–Indian strain) 100–500 TCID50 were added to strike the surveying cells. The IFN titer was determined as the reverse counts of the highest dilution which inhibited more than 50% of cytopathological changes as compared with controls. Standard α–IFN and γ–IFN were used for correction and calculated into IU / ml. The results were listed in Tab 3. PSP 10–1000 μg / ml increased α–IFN to 8000–16000 IU / ml, i.e. 4–8 times higher than that of classical group or 2–4 times higher than that of the priming group. The effect of PSP revealed a dose–dependent relationship. The titer of α–IFN was not reduced after acidified, neutralized or 56℃ denatured, indicating that it is an acid and heat stable α–IFN. γ–IFN titer in PSP (10–1000 μg / ml)–treated super–natants was 4 times higher than classical groups and 2 times higher than that of priming groups. The same results were obtained after repeating the experiments 4 times. It is therefore concluded that PSP has the ability to induce IFN–α and γ production.

Tab 3. Effect of PSP on interferon induction from human peripheral leukocytes. The experiment was repeated 4 times and the results were all the same. Interferon titers were calculated as IU / ml × 10^{-3}.

	Classical[a] group	Priming[b] group	PSP (μg / ml) group[c] 1	10	100	1000
IFN–α	2	4	8	16	16	16
IFN–γ	1	2	2	2	4	4

a: neither drug nor priming interferon was added into the cultures.
b: interferon (200 IU / ml) was added in the WBC cultures and preincubated at 37℃ for 1 h.
c: PSP were added into human WBC suspensions and incubated for 24 h.

4 Effect of PSP on T cell–mediated delayed type hypersensitivity (DTH) reaction in mice 1% Dinitrofluorobenzene (DNFB) was dissolved in acetone oil (1:1) solution just before use. 1% DNFB solution 50 μl was spread on the shaved abdominal skin (3 × 3 cm^2) of C57BL mice on d 0. PSP 50 mg / kg ip daily from d1–5. On d 5, 1% DNFB 10 μl applied on the right ear, while the left one was used as control. After 24 h, 8–mm diameter ear pinna was taken from both ears. The weight difference between right and left ear pinna was considered as the degree of DTH reactions. Results were shown in Tab 4. PSP alone had no effect on DTH response. Cyc inhibited DTH response and thymus index ($P<0.01$). When mice received PSP and Cyc together, the inhibition of Cyc on DTH was no longer seen and DTH response restored to normal.

Tab 4. Effect of PSP ip into mice on dinitrofluorobenzene (DNFB)–induced delayed–type–hypersensitivity (DTH) response. $n=8$, $\bar{x}\pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ vs control. $^{+}P>0.05$, $^{++}P<0.05$ vs Cyc group.

	mg / kg	DTH (R–L)	Spleen index	Thymus index
Control	––	11.0 ± 3.4	59.7 ± 9.8	14.5 ± 4.2
PSP	25	13.3 ± 5.1*	61.0 ± 8.6*	16.8 ± 2.8*
Cyc	25	7.9 ± 2.3**	46.9 ± 4.8*	8.1 ± 2.0***
PSP+Cyc	25+25	13.0 ± 4.1^{++}	59.2 ± 7.5^{+}	9.6 ± 4.0^{+}

5 Effect of PSP on clearance rate of iv charcoal particles in mice PSP 50 mg / kg was injected ip daily to ICR ♂ mice for 4 d. Indian ink (Winsor & Newton product, England 1:5 diluted) was injected iv 10 ml / kg. Punctually at 2 and 20 min after iv, 20 μl of blood were taken from retro–orbital vein and dissolved into 2 ml Na_2CO_3 0.1 % solution. Absorbance was measured at 680 nm. Clearance index K and phagocytic index α were calculated as previously reported[6]. PSP 50 mg / kg elevated both K values (control group 0.018 ± 0.004, $n=14$; PSP group 0.028 ± 0.007, $n=18$. $P<0.01$) and α value (4.15 ± 0.44 to 4.74 ± 0.56, $P<0.05$), indicating that it increased the phagocytic function of host reticulo–endothelial system.

DISCUSSION

The authors reported before that PSP may prevent the tumor–induced immuno–deficiency and potentiate antibody formation in normal and tumor–bearing mice[3]. This paper showed further that PSP increased the production of IL–2 and IFN and the phagocytic action of reticulo–endothelial system. Since natural killer cells play an important role in host defence, the anti–tumor effect of PSP may be mediated through increasing production of IL–2 and IFN which then augment cytotoxic activity of natural killer cells synergetically[7]. The immune–enhancing actions of PSP on immunosuppressed mice caused by CY are more evident than that on normal mice. The effects of PSP is more significant when given *in vivo* than that *in vitro*. These results all suggest that PSP is a host–mediated biological response modifier.

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云芝多糖肽的免疫增强作用

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提要 由彩绒革盖菌(*Coriolus versicolor*)中提取的云芝多糖肽(PSP)在浓度为 100–800 μg / ml 时能引起淋巴细胞明显增殖. 小鼠 ip 环磷酰胺 25 mg / kg 抑制了活化 T–细胞产生 IL–2 和 T 细胞中介的 DTH 反应, 如同时给予 PSP 25 mg / kg 连续 5 天, 可以对抗以上的免疫抑制效应. PSP 浓度为 10～1000 μg / ml 时可使人白细胞产生 α–干扰素和 γ–干扰素的能力较空白对照组分别提高 8 倍和 4 倍. 此外, PSP 还能增强网状内皮系统的吞噬功能. 以上结果提示 PSP 的抗癌作用可能与其增强宿主的免疫功能有关.

关键词 彩绒革盖菌; 多糖肽; T 淋巴细胞; 白细胞介素; 环磷酰胺; 干扰素类 I 型; 干扰素类 II 型; 迟发型超敏感性

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