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Effects of *Tremella* polysaccharides on immune function in mice[1]

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ABSTRACT It was found *in vitro* that *Tremella* polysaccharides (TP) (50, 100, 150 and 200 μg/ml) augmented lymphocyte proliferation induced by Con A and did not antagonize the suppressive effect of hydrocortisone on lymphocyte proliferation. *In vivo* TP promoted the plaque-forming cell (PFC) response to SRBC in mice. TP 50 and 100 mg/kg ip for 5 d produced 77.6% and 81.8% increases in PFC response respectively. At the doses of 150 and 200 μg/ml, TP decreased the interleukin 2 (IL-2) activities in the supernatant of culture media of mouse spleen cells. TP (50 μg/ml) enhanced the lymphocyte proliferation induced by Con A and increased the PFC response to SRBC by 47.1 % in 14-month-old mice.

KEY WORDS *Tremella fuciformis*; polysaccharides; lymphocytes; hydrocortisone; concanavalin A; plaque assay; interleukin 2

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Tremella polysaccharides (TP) are important components isolated from *Tremella fuciformis* Berk. TP showed antitumor activity in mice[1,2]. Our previous researches demonstrated that TP increased phagocytosis of intraperitoneal macrophages and production of hemolysin in normal and immunosuppressive mice[3,4].

In order to further observe the effects of TP on immune function in mice, we examined the effect of TP on the lymphocyte proliferation induced by Con A in normal mouse spleen cells and immunosuppressive mouse spleen cells caused by hydrocortisone and the effect of TP on humoral immunity with plaque forming cell response. We also examined the effect of TP on the interleukin 2 (IL-2) production

in mice. Moreover, we studied the restorative effect of TP on the cellular and humoral immune function in aged mice.

MATERIALS

TP was isolated from the fruiting body of *Tremella fuciformis* Berk which was harvested in Fujian Provice by sequential hot water and ethanol extraction. It is a kind of polysaccharides containing protein provided by Sanming Mycological Institute. The polysaccharides consisted of mannose, glucuronate, xylose, fucose and glucose. Its molecular weight was 300 000. TP dissolves easily in water. Concanavalin A (Con A) was purchased from Sigma Co.

C 57 BL/6 J ♂ and ♀ mice, aged 2–3 months. Swiss ♂ and ♀ mice, aged 2–3 and 14 months respectively.

METHODS AND RESULTS

Effect of TP on the lymphocyte proliferation induced by Con A in mice Con A (2 μg/ml) induced lymphocyte proliferation was measured by radioactivity of [^{3}H]TdR incorporation into the mouse spleen cells from C 57 BL/6 J mice[5,6].

Fig 1 indicated that TP 50, 100, 150 and 200 μg/ml increased the Con A–induced proliferation of lymphocytes by spleen cells from C 57 BL/6 J mice aged 2–3 months. The correlation coefficient was 0.96.

Effect of TP on the inhibitory activity of hydrocortisone (HC) in mouse lymphocyte proliferation induced by Con A The experimental method was the same as the above but microplate wells also received certain dose of HC.

Fig 2 shows that, at the doses of HC 0.025–1.0 μg/ml, the inhibitory effects increased in a concentration–dependent manner. The inhibitory effect by 0.2 μg/ml approached the maximum.

Tab 1 expresses the effects of TP on

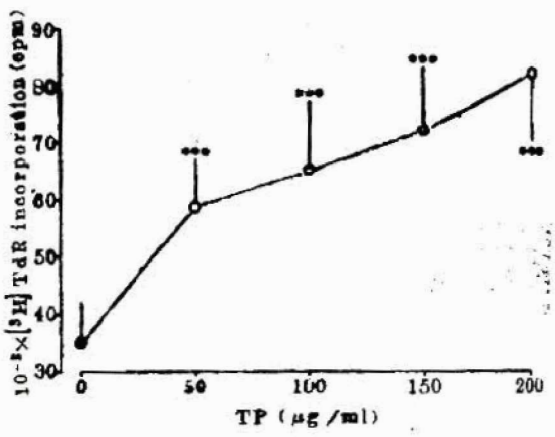

Fig 1. Effect of *Tremella* polysaccharides (TP) on the lymphocyte proliferation induced by Con A in 2–3 month aged mice. $n=12$, $\bar{x}\pm SD$. ***$P<0.01$.

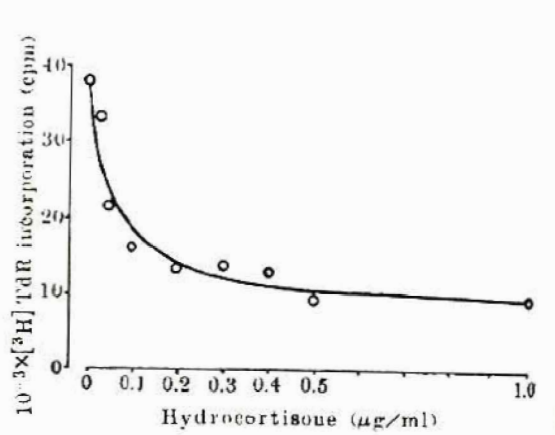

Fig 2. The inhibitory effect of hydrocortisone on the lymphocyte proliferation induced by Con A, $n=3$.

the inhibitory activities of HC in mouse lymphocyte proliferations induced by Con A. At the doses of 25, 50, 100 and 150 μg/ml, TP did not antagonize the suppressive effects of HC. When the concentration of HC was reduced to 0.1 μg/ml, TP did not show the antagonistic effect either.

Effect of TP on the lymphocyte proliferation induced by Con A in mice aged 14 months The lymphocyte proliferation response of 14–month–old mice was lower than that of 3–month–old mice (Tab 2). TP 50 μg/ml promoted lymphocyte prolif-

Tab 1. Antagonistic effect of *Tremella* polysaccharides (TP) on the inhibitory activity of hydrocortisone (HC) in mouse lymphocyte proliferation induced by Con A. $\bar{x}\pm SD$. $^{*}P<0.05$ *vs* HC group. $^{\dagger\dagger}P<0.05$ *vs* control.

TP (μg/ml)	HC	Sample number	[^{3}H]TdR incorporation (cpm)
–	–	6	37 388±15 226
–	0.2	3	13 401±833††
25	0.2	6	11 753±2727*
50	0.2	6	12 979±1560*
100	0.2	6	17 204±2711*
150	0.2	6	14 265±1776*

eration induced by Con A in 14-month-old mice. TP 100 μg/ml had no distinct effect on it, but TP 150 and 200 μg/ml inhibited lymphocyte proliferation response in 14-month-old mice. These findings indicated that the optimal dose of TP in aged mice was less than that in young mice.

Tab 2. Effect of TP on the lymphocyte proliferation induced by Con A in 14 month aged mice. $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* aged control. $^{\dagger\dagger\dagger}P<0.01$ *vs* young control.

Age (month)	TP (μg/ml)	Sample number	[^{3}H]TdR incorporation (cpm)
3	0	6	50 079±7 552
14	0	5	36 812±2 690†††
14	50	6	40 943±1 726**
14	100	6	34 285±4 889*
14	150	6	30 690±4 802**
14	200	6	24 720±6 316***

Effect of TP on the plaque forming cell (PFC) response to SRBC in mice Swiss mice aged 2–3 months were divided into 3 groups and medicated according to Tab 3. The mice in control group were given equal volume of normal saline. Mice were immunized by ip 10% sheep red blood cells (SRBC) 0.2 ml. After 4 d, IgM PFC was measured by the plaque technique of Cunningham[7]. The spleen cell suspensions were mixed with SRBC and complement. The mixtures were incubated at 37℃ for 1 h, and PFC was measured.

Tab 3. Effect of TP on the plaque-forming cell (PFC) response to SRBC in mice aged 3 and 14 months. $n=6$, $\bar{x}\pm SD$. $^{***}P<0.01$ *vs* YC (Young control). $^{\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.01$ *vs* AC (Aged control).

Age (month)	Treatment	Dose(ip) (mg/kg·d)	PFC/10^6 spleen cells
2	YC	—	1504±420
2	TP	50×5	2671±699***
2	TP	100×5	2734±772***
3	YC	—	1910±473
14	AC	—	995±182***
14	TP	50×5	1464±244†††
14	TP	100×5	1148±491†

TP 50 and 100 mg/kg promoted markedly the PFC response to SRBC in mice.

Effect of TP on the PFC response to SRBC in mice aged 14 months The experimental method was the same as the above test. PFC response of 14-month-old mice was lower than that of 3-month-old mice (Tab 3). TP 50 mg/kg increased PFC response of 14-month-old mice, but TP 100 mg/kg showed no significant effect on it. The optimal dose of TP in aged mice was less than young mice.

Effect of TP on the IL-2 production in mice Interleukin 2 activity was assayed by titration of mouse thymus cell[8,9].

TP 50 and 100 μg/ml exerted no distinct effect on IL-2 activity (Tab 4). TP 150 and 200 μg/ml decreased the IL-2 activity.

Tab 4. Effect of TP on interleukin 2 (IL-2) production in mice aged 2–3 months. $n=7$, $\bar{x}\pm SD$. $^{*}P>0.05$, $^{**}P<0.05$.

TP(μg/ml)	IL-2 activity (U/ml)
0	23.0±4.0
50	22.1±4.2*
100	24.4±6.6*
150	18.0±1.8**
200	17.2±3.2**

DISCUSSION

TP *In vitro* significantly increased the Con A-induced proliferation of mouse spleen cells. The results indicate that TP promotes body cellular immune function. However, TP did not show to antagonize the suppressive effect of hydrocortisone on lymphocyte proliferation *in vitro*. It may be relating to that TP didn't affect or decreased the IL-2 activity because lymphofactors were considered as the action focus of glucocorticoid.

PFC is a method to examine single antibody forming cells *in vitro*. It is a specific index to study the effect of medicine on body humoral immune function. Our results showed TP promoted the PFC response to SRBC in mice. Our previous researches indicated that TP increased the production of hemolysin in mice immunized with SRBC. It also increased the production of hemolysin in immunosuppressive mice induced by cyclophosphamide[4]. Take these findings together, we may consider that TP increases the production of antibody through increasing PFC.

We also proved that, comparing with 3-month-old mice, T cell proliferation induced by Con A and PFC response to SRBC decreased clearly in 14-month-old mice. TP enhanced these reactions in 14-month-old mice, so TP can restore lower cellular and humoral immune function of old animal.

At the optimal dose, the immune modulation agents promote body immune functions. They do not bring into full play when dose is too little, while their actions reduce when dose is too large. From our results, it seems that the optimal dose of TP for PFC response in old animals is less than that of young animals. It may be attributed to the decreases of biotransformation of drugs in senile body.

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