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Immunostimulating Activity of *Phellinus linteus* Extracts to B-lymphocyte

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Abstract □ *Phellinus linteus* was examined on its immunostimulating activities using an *in vitro* immunization and plaque forming cell assay. When lymphocytes were exposed to the extract of *Phellinus linteus*, the number of antibody forming cell was increased. In *in vitro* plaque forming cell assay, the immunostimulating effect was about 4.8 and 5.0 times of unimmunized control in polyclonal and T-independent antibody response, respectively. Especially, *Phellinus linteus* significantly increased the antigenicity of TNP-LPS used as T-independent antigen. But *Phellinus linteus* did not show a mitogenic effect on B-lymphocytes. These results suggest that immunostimulating activity of *Phillinus linteus* might be associated with a funtional stimulation of B-lymphocyte involved in humoral immune responses.

Keywords □ *Phellinus linteus*, antibody-forming cell polyclonal antigen, T-independent antigenic effect.

To find a new immunomodulating drug from natural products, many mushrooms were investigated for several decades. Roland *et al.* found a new antitumor agent, calvacin from *Calvatena gigantea*[1]. Gregory and co-workers evaluated antitumor activities of Basidiomycetes on sarcoma 180, mammary adenoma 755 and leukemia L1210[2]. Antitumor compounds from Basidiomycetes were identified as mainly polysaccharides and protein-bound polysaccharides. These polysaccharides had quite a different mode of action from nonselective antitumor agents and showed their antitumor activities by potentiating the immune systems without a direct cytotoxicity. In previous reports, several kinds of immune parameters were introduced to determine the pharmacological mechanism of the effects of polysaccharides from Basidiomycetes in immunomodulation. For cell-mediated and natural immunity, natural killer cell (NK), cytotoxic T lymphocyte (CTL) and macrophage assay were performed. In most cases, polysaccharide which showed an antitumor activity on transplanted tumor cells stimulated cell-mediated and natural immunity[3-5]. To evaluate humoral immunity, plaque forming cell (PFC) assay was done in tumor-bearing and normal mice. Usually, polysaccharide compounds increased antiboty-forming cells (AFCs) in normal mice and recovered antibody production suppressed in tumor-bearing mice when lymphocytes were immunized in *in vivo* with sheep red blood cell (sRBC)[6,7]. *Pellinus linteus* was also examined in a few immunological studies. NK cell activity was stimulated and the suppressed T-lymphocyte was recovered in tumor-challenged mouse by an oral administration of *Phellinus linteus* extract. AFC induced by an *in vivo* T-dependent antigen (sRBC) was also increased by i.p. injection of *Phellinus linteus* polysaccharide to normal mouse[8]. But the exact mechanism on the immunopotentiation and antitumor activity of *Phellinus linteus* was not elucidated. In present paper, the stimulation effects of *Phellinus linteus* extract on the *in vitro* polyclonal and T-independent antibody

response were determined. An *in vitro* immunopharmacological parameter was also selected to support the further purification and characterization of active compounds in *Phellinus linteus* extract.

EXPERIMENAL METHODS

Materials

Water extract of *Phellinus linteus* was prepared from artificially cultivated mycelia. Female (C57BL/6×C3H)F1 (B6C3F1) mice maintained in GERI, KIST, were used in all experiments. The mice were used as the source of the spleen cells when they were 17-20g in weight. Sheep red blood cells were obtained from Korea Media Co., Ltd. (Seoul, Korea). Guinea pig complement and RPMI 1640 were purchased from GIBCO BRL (Grand Island, NY, USA).

In vitro antibody response

Spleen cell suspensions were obtained using the method described by Kim *et al.*[9] The spleen cells were resuspended in RPMI 1640 with 10% fetal calf serum and adjusted to 5×10^6 cells/m*l* for the polyclonal response to lipopolysaccharide (LPS) and to 1×10^7 cells/m*l* for the T-independent response to TNP-LPS. The cultures (0.5 m*l* aliquot well ; 4 replicate wells/treatment group) were set up in individual wells of a 48-well cluster plate (Costar) and LPS (25 μg/m*l*; Sigma Chem. Co.) or TNP-LPS (5 μg/m*l*) was added. 2-Mercaptoethanol (Sigma) was added to all cultures at a final concentration of 5×10^{-5} M. For all experiments, the antibody plates were incubated with rocking (8-10 rocks/min) at 37℃ in Bellco (Bellco Biotech., Vineland, NJ, USA) stainless-steel tissue culture boxes in an atmosphere of 10% CO_2, 7% O_2 and 83% N_2 at 4-5 psi. The polyclonal antibody response was measured on day 2 and T-independent antibody response was measured on day 3. AFCs were enumerated using a modified Jerne plaque assay and cell number was counted by using haemacytometer as previously described[9].

RESULTS AND DISCUSSION

Phellinus linteus extract showed a similar action with antigens which selectively immunized B-lymphocytes. Fig. 1 shows the effect of *Phellinus linteus* extract on the *in vitro* antibody response to LPS.

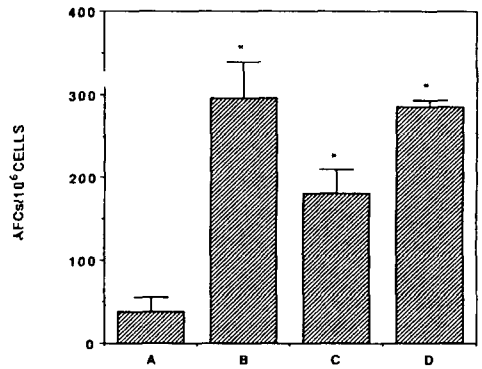

Fig. 1. Effect of *Phellinus linteus* extract on the *in vitro* polyclonal antibody response.
A: unimmunized control, B: lipopolysaccharide (LPS; 25 μg/m*l*), C: *Phellinus linteus* extract (1 mg/m*l*), D: LPS and *Phellinus linteus* extract. Values represented the mean± SD of triplicate cultures.
*indicates $p<0.05$, when compared to the control values.

LPS (25 μg/m*l*) showed a strong immunization and AFCs were increased to about 7.8 times of unimmunized control group. At 1 mg/m*l*, *Phellinus linteus* extracts had a similar effect with LPS. About 5 times increment of AFCs was produced against TNP-haptenated sRBC by *Phellinun linteus* extract. The presence of *Phellinus linteus* extract in the medium did not affect the induction profile of AFCs by LPS alone. The effect of *Phellinun linteus* extract on the T-independent antibody response to TNP-LPS is shown in Fig. 2. In the response to TNP-LPS, *Phelliun linteus* extract and TNP-LPS showed an exact additive effect. AFCs were increasled to 7.2 and 4.8 times of unimmunized control group by the treatment of TNP-LPS (5 μg/m*l*) and *Phellinus linteus* extract (1 mg/m*l*), respectively. By a simultaneous treatment of TNP-LPS and *Phellinus linteus* extract, about 13.4 times of AFCs of unimmunized control group were produced. In view of the above results, *Phellinus linteus* extract might be assumed as a B-lymphocyte antigen which showed a similar mechanism with LPS and caused an stimulation of immunization by TNP-LPS. The mitogenic effect of *Phellinus linteus* extract was determined at a concentration of 1 mg/m*l* (Table I). When treated alone or in combination with other B-lymphocyte antigens, *Phellinus linteus* extract did not

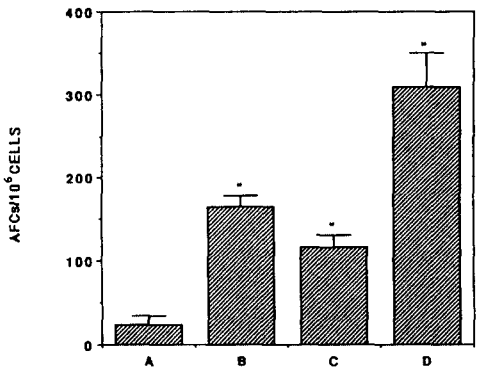

Fig. 2. Effect of *Phellinus linteus* extract on the *in vitro* T-independent antibody response.
A: unimmunized control, B: TNP-LPS (5 μg/ml), C: *Phellinus linteus* extract (1 mg/ml), D: TNP-LPS and *Phellinus linteus* extract. Values represented the mean± SD of triplicate cultures. *indicates $p<0.05$ when compared to the control values.

Table I. Effect of *Phellinus linteus* on viable cell number in the *in vitro* polyclonal and T-independent antibody response

Treatment	Polyclonal ($\times 10^4$ cells/well)	T-independent ($\times 10^4$ cells/well)
Control	24.6± 0.5	54.0± 1.0
LPS (or TNP-LPS)	27.7± 1.9	56.3± 2.4
P. linteus	30.7± 1.7	57.0± 1.2
LPS (or TNP-LPS) +*P. linteus*	28.7± 1.5	53.3± 4.6

Values represented the mean± SD of triplicate cultures.

show a mitogenic effect. These results suggest that *Phellinus linteus* might cause an immune stimulation which was not related with mitogenic effect. The stimulation effect of *Phellinus linteus* extract could be observed by itself and in combined treatment with other antigens. *Phellinus linteus* was reported to show a strong antitumor activity and immunostimulating effect on NK cell and T-lymphocytes. Recently, the stimulation effect of *Phellinus linteus* polysaccharide on humoral antibody formation was determined in the *in vivo* T-independent immunization[8]. In present *in vitro* antibody response, it was shown that *Phellinus linteus* extract could act as a polyclonal antigen similarly with LPS. The results and method reported in this study would contribute to the pharmacological and chemical studies on *Phellinus linteus* for the development of an immunomodulating agent.

LITERATURE CITED

1. Roland, J. F., Chmielewicz, Z. F., Weiner, B. A., Gross, A. M., Boening, O. P., Luck, J. V., Bardos, T. J., Really, H. C., Sugiura, K., Stock, C. C., Lucas, E. H., Byerrum, R. U. and Stevens, J. A.: Calvacine, a new antitumor agent. *Science* **132**, 1987 (1960).
2. Gregory, F. J., Healy, E. M., Agerbory, H. P. K., Jr. and Warren, G. H.: Studies on antitumor substances produced by *Basidiomycetes*. *Mycolilgia* **58**, 80 (1966).
3. Dennert, G. and Tucker, D.: Antitumor polysaccharide lentinan A T cell adjuvant. *J. Natl. Can. Inst.* **51**, 1727 (1973).
4. Ortaldo, J. R. and Longo, D. L.: Human natural lymphocyte effector cell: Definition, analysis of activity, and effectiveness. *J. Natl. Can. Inst.* **80**, 999 (1988).
5. Nielsen, H. J., Moesgaard, F. and Kehlet, H.: Ranitidine for prevention of postoperative suppression of delayed hypersensitivity. *Am. J. Surg.* **157**, 291 (1989).
6. Kikuo, N., Chikao, Y. Kenichi, M. Takayoshi, F. and Kenji, T.: Restoration of antibody-forming capacities by PS-K in tumor bearing mice. *Gann.* **66**, 365 (1975).
7. Ryuzo, O., Shozo, Y., Kikuko, W., Sayoko, S., Kuniyuki, I. and Kazumasa, Y.: Effect of protein-bound polysaccharide preparation, PS-K, on the immune response of mice to sheep red blood cell. *Gann*, **67**, 97 (1976).
8. Chung, K. S., Kim, S. S., Kim, K. Y., Kwak, Y. S., Kim, H. S., Kim, K. H. and Han, M. W.: Immunomodulating activity of Kp, antitumor protein-polysaccharide fraction of *Phellinus linteus*. *Proceedings of the 41st Annual Convention of the Pharamaceutical Society of Korea*, p. 105 (1992).
9. Kim, D. H., Yang, K. H., Johnson, K. W. and Holsapple, M. P.: Suppression of *in vitro* antibody production by dimethylnitrosamine in mixed cultures of mouse primary hepatocytes and mouse splenocytes. *Toxicol. Appl. Pharmacol.* **87**, 32 (1987).