Hematopoietic Effect of *Phellinus linteus* Polysaccharide in Mouse Splenocytes and Bone Marrow Cells

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**Abstract** — In anti-cancer therapies, radiotherapy and chemotherapy show a superior inhibition effect on cancer cell growth, but those are very toxic to normal tissues and organs. Particularly, drugs for neutropenia, one of chemotherapy agents, which suppress the function of bone marrow, are needed to be controlled in terms of their dosage and therapy period because of their side effect. *Phellinus linteus* polysaccharide (PL) has been reported to increase the number of splenocytes and bone marrow cells. PL has been shown to decrease the side effects of cyclophosphamide (CYC) treatment to the cancer patients. PL showed no effects in semisolid clonogenic assay, suggesting that PL doesn’t contain substantial compounds to substitute for colony stimulating factors (CSFs). On the other hand, PL increased the expression of SCF, IL-3, GM-CSF, TPO genes. These results indicate that PL may promote the growth and proliferation of splenocytes and bone marrow cells through indirect or CSFs-dependent pathway, which may lead to a hematopoiesis.

**Keywords** — hematopoiesis, *Phellinus linteus* polysaccharide, splenocytes, bone marrow, colony stimulating factors

**INTRODUCTION**

Cancer has been considered as one of incurable diseases, and a lot of researches have focused on curing cancers. Surgery, radiation therapy and chemotherapy have been considered as the major therapeutic modalities commonly used for the treatment of a variety of cancer patients. However, in many cases, those therapeutic modalities induce several side effects such as suppression of bone marrow functions, decrease of immune functions, abnormalities in digestive functions, and weakness (Ito et al., 1979). Particularly, side effects of cancer therapies at bone marrow functions such as decrease of granulocytes could induce the death of the cancer patients due to the infection and septicaemia (Chauvergne et al., 1996; Pronk et al., 1995; Cascini et al., 1995). Therefore, therapeutic periods and the doses of the chemotherapy should be altered in order to maintain the appropriate number of neutrophils, which indicate the decrease of granulocytes. Recently, immunotherapy, inductor of differentiation and cytokines, has been attracted in cancer therapies. Especially, colony stimulating factors which regulate the growth of bone marrow cells have been employed in clinics (Clark et al., 1987; Bociek et al., 1996; Bokemeyer et al., 1996). However, the application of the recombinant colony stimulating factors to the cancer patients has been restricted due to the side effects on the stimulation of cancer cell growth or toxicity against human being (Nagleri et al., 1998; Ridolfi et al., 1998).

Hematopoiesis is the process which hematopoietic stem cells grow and divide into blood cells. In human, this process carried out in liver and spleen for fetus and in bone marrow for adults. In case of mice and other animals, it occurs in spleen (Dexter et al., 1997). The generation of blood cells is known to be regulated by the direct contact of cells to one another, various hematopoietic stimulating factors and cytokines, but this process is not fully elucidated yet. The hematopoietic stimulating factors not only are essential for the growth and differentiation of blood cells, but also affect on their survival. It has been also reported that the hematopoietic cells undergo the apoptosis without hematopoietic stimulating factors (Jan et al., 1991). The examples of hematopoietic factors are granulocyte/macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor (SCF), thrombopoietin (TPO), erythropoietin (EPO), interleukin-1 (IL-1), interleukin-3 (IL-3), and those factors are known to promote the growth and the differentiation of blood cells to specific types of blood cells by affecting bone marrow cells (Brugger et al., 1994; Arai et al.,...
Hematopoietic Effect of Phellinus linteus Polysaccharide

Laboratory animals and materials

Male ICR mouse were purchased from HanLim laboratory and allowed to acclimatize in our facility for one week. The animals were breeding under the conditions of 24±2°C, controlled light, and feeding without antibiotics. Phellinus linteus polysaccharide fraction was offered as 500 µg/mL cyclophosphamide (CYC) was purchased from Sigma and diluted to 1.6 µg/mL. All reagents were diluted with 10% FBS RPMI 1640 for splenocytes and 20% FBS alpha MEM for bone marrow cells.

MTT assay

Spleen and thighbone were obtained from mouse sacrificing by enucleating. Each splenocytes and bone marrow cells were separated from these removed tissues. Cells were plated into 96 well culture plates at 1×10⁶ cells per well and were treated 500 µg/mL PL, 1.6 µg/mL CYC, or 500 µg/mL PL plus 1.6 µg/mL CYC. The plates were incubated for 48 hr at 37°C in a humidified atmosphere of 5% CO₂. Thereafter, each well was treated with 10 µl of methylthiazoletetrazolium (5 mg/ml MTT). In order to dissolve the formazan crystals (which is result of previous step), each plate was added 10% SDS containing 0.01 N HCl. The optical density was read on a micro plate reader at 570 nm.

Semisolid clonogenic assay

Splenocytes or bone marrow cells resuspended in semisolid medium as 2×10⁶ cells/ml concentration were cultured in 35 mm petri dishes. Semisolid medium was containing Iscove’s modified Dulbecco’s medium (IMDM) and 1% agar, 20% fetal bovine serum and 1.6 µg/mL CYC, 500 µg/mL PL, or 1.6 µg/mL CYC plus 500 µg/mL PL according to sample group. Cell cultures were incubated for 9 days at 37°C in a humidified atmosphere with 5% CO₂. Colonies were counted with a microscope.

RT-PCR (reverse transcription - polymerase chain reaction)

Total RNA was extracted from 10⁶ cells using Trizol reagent (Life Technologies, Inc.-BRL, Gaithersburg, MD) according to the protocol provided by the manufacturer and quantified by spectrophotometer (260 nm), cDNA were synthesized from 1 µg of DNA-free RNA, added to the reverse transcriptase (RT) mixture. In the thermal cycler, tubes were heated at 42°C for 60 minutes, 94°C for 5 minutes, quickly chilled on ice to 4°C. The RT reaction product was amplified by PCR using specific primers. Primer sets included the SCF primers 5’-TAACCTCT-CAACTATGTCGCC-3’(forward) and 5’-CGTTTACAGCTTCAGTTCC-3’(reverse), and IL-3 primers 5’-GAAGTGGCATC-CTGAGGACAGATACG-3’(forward) and 5’-GACCCATGGAGTTTCC-3’(reverse), and TPO primers 5’-ATGAGTTCCATTCAC-3’(reverse). PCR was begun with a denaturing step of 94°C for 5 minutes, then 34 cycles of 94 for 30 seconds, 55°C for 60 seconds, and 72°C for 60 seconds. For analysis, reaction product was run in 1.8% agarose gel.

Statistical analysis

All results are given as mean±S.D. Comparison between means was made by Student’s t test

RESULTS

Effect of PL on proliferation of splenocytes and bone marrow cells

To confirm whether treatment of PL increased the proliferation of hematopoietic cells, the number of splenocytes and bone