

## Investigation of Immunomodulating Activity of the Medicinal Mushroom *Flammulina velutipes* (Curt.: Fr.) P. Karst. *In Vitro*. Cytokine Induction by Fruiting Body Extract

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Recently focused attention on the development of immunotherapy, as well as on the discovery of new immune-potentiators, significantly increases the interest in Higher macroscopic fungi as a natural source of biologically active substances (BASs). The U.S. National Cancer Institute has classified many of the isolated and identified BASs of Medicinal Mushrooms (MMs) as antitumor chemicals with immune-modulating activity. Today, several antitumor polysaccharides and their protein complexes have been separated from MMs, such as *Ganoderma lucidum* (Cust.: Fr.) P. Karst., *Trametes versicolor* (L.: Fr.) Lloyd, *Schizophyllum commune* Fr.: Fr. and *Lentinus edodes* (Berk.) Sing. Among the MMs the *Flammulina velutipes* (Curt.: Fr.) P. Karst. plays a special role. It has been extensively investigated in regard to its immune activity. The commercially developed antitumor, immunomodulating and antiinflammatory mushroom's products from this species were separated. Preliminary data concerning the antifungal, antiviral (including anti-HIV), host-mediated antitumor and immune-regulating activities of *F. velutipes* were also reported. The water-soluble (1→3)- $\beta$ -D-glucan-protein complex and Proflamin, an antitumor, water-soluble glycoprotein were isolated from fruiting body and submerged cultured mycelial biomass of *F. velutipes*, respectively. They are regarded as biological response modifiers.

The fungal glucans and glucan-protein complexes are not directly toxic to tumor cells. They inhibit tumor growth by stimulating and activating the host's immune system and macrophage

activity with or without interferon induction. They can augment the induction of antigen-specific cytotoxic T lymphocytes, macrophages, and other nonspecific immune responses. However, most of the mushroom-generated antitumor substances and a detailed mechanism of their action have not been clearly defined yet.

In our experiment, the effect of fruiting body extract of *F. velutipes* on cytokine induction [interleukin (IL) -2 and -12, interferon (IFN) - $\alpha$  and - $\gamma$ ] on human peripheral blood mononuclear cells (PBMCs) was investigated. In the screening process the four donors (two healthy, one with immune deficiency, another with tongue cancer) were included. The PBMCs were separated by Boyum's method. The fruiting body was extracted with EtOH. Then, the fungal extract obtained was diluted with Eagle's solution (1:20) and added to PBMCs at 1 mcg/ml. As a simple inducer for IL-2 and IFN- $\gamma$ , the phytohemagglutinin (PHA) was also used. It was added to PBMCs at 15 mcg/ml. The incubation was carried out in the dark at 37°C during 48 hr. Cytokine induction *in vitro* was examined by enzyme-linked immunosorbent assay (ELISA).

The preliminary results revealed that extract of *F. velutipes* raised the level of IL-2 (T-cell growth factor) in comparison with the PHA induction effect in all donors. After incubation with fungal extract, the average positive level of IL-12 and IFN- $\alpha$  induction was also observed. The IL-12 stimulates the production of INF- $\gamma$  from T cells and natural killer (NK)-cells, initiating cell-mediated immunity. In the tested donors, a low level of IFN- $\gamma$  induction was observed.

Thus, the fruiting body extract of *F. velutipes* stimulates the host's immune system and macrophage activity with interferon induction. The *F. velutipes* definitely possesses immune-stimulating activity, which is expressed by the

enhancement of cytokine (IL-2, IL-12 and INF $\alpha$ ) levels in human PBMCs.

Purposeful further investigation of anti-tumoral, antiviral, antibacterial, and antioxidant activity of *F. velutipes* is in progress.