

## Induction of Immunomodulatory Cytokine Gene Expression by *Ganoderma lucidum* (Curt.: Fr.) P. Karst. Polysaccharide in the Mouse

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The immunomodulatory action of an anti-tumor polysaccharide isolated from *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (designated GLP) was elucidated through analyzing the expression profile of cytokines induced by GLP in mice. Many mushroom polysaccharides are shown to be antitumor and immunomodulating agents, and their mode of immunomodulation generally has been proposed as a potentiation of the various immune responses through the regulation of cytokines in the cytokine network of the body.

GLP was isolated by hot aqueous extraction and ethanol precipitation from fruiting bodies of *Ganoderma lucidum*. It showed no direct anti-proliferative effect as evaluated *in vitro* using several cancer cell lines, such as human promyelocytic leukemia HL-60, myelogenous leukemia K-562, breast cancer MCF-7, lung cancer SPC-A, and hepatoma SMMC-7721 at the doses 50–200 µg/ml of culture medium. When GLP was administered intraperitoneally into the Sarcoma 180 tumor-bearing BALB/c mice at a dose of 20 mg/kg/day for 10 consecutive days, it significantly suppressed *in vivo* the growth of Sarcoma 180 solid tumor, and thus exhibited prominent anti-tumor activity.

The immunomodulatory effect of GLP through the *in vivo* induction of gene expression of cytokines in the mouse was studied using primers of specific cytokines and reverse

transcription-polymerase chain reaction (RT-PCR). Total RNA samples were isolated from the splenocytes and peritoneal exudate cells (macrophages) from the male inbred BALB/c mice that had received daily intraperitoneal injection of 20 mg/kg for 5 consecutive days, and those from the untreated mice were used as the control. Seven out of the seventeen cytokine mRNAs were detected in the splenocytes and peritoneal exudate cells from the control and treated mice. Among the seven detectable cytokine genes, GLP induced a marked increase in the expression levels of interleukin (IL)-1 $\alpha$  (2-fold), IL-1 $\beta$  (3-fold), tumor necrosis factor (TNF)- $\alpha$  (2-fold), and TNF- $\beta$  (2.2-fold). The mRNA level of TGF- $\beta$  in the splenocytes of tumor-bearing mice was only slightly higher than that of the normal control mice. The expression levels of SCF and M-CSF remained the same as in the control or slightly down-regulated. These results indicated that the induction of gene expression of cytokines by anti-tumor polysaccharides seemed quite complicated. Therefore, kinetic experiments of gene expression of cytokines and cytokine receptors might be important to fully characterize the gene expression of polysaccharide-mediated cytokines and the immunomodulatory action.

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