〈총설1〉

Immunological Activities and Characteristics of GLG Isolated from the Mycelium of *Ganoderma lucidum* IY009

Lee, June-Woo and Sang-Mo Kang*

Biotechnology Lab., Central Institute of Research, IL Yang Pharmaceutical Co. Ltd.,
*Department of Microbiological Engineering, KonKuk University

In order to develope the new polysaccharides having antitumor activity, many kinds of *Ganoderma lucidum* were collected from various regions in Korea and classified by comparing each isozyme pattern. Antitumor glucan, named GLG(*Ganoderma lucidum* glucan; GLG) was isolated from the fermentation broth of *Ganoderma lucidum* IY009 and investigated the physico-chemical properties, antitumor activity, anticomplementary activity, change of a specific protein, biological activity of macrophage and proliferation of the immunocytes. Simiarity of isozyme band patterns among the isolates of *Ganoderma lucidum* IY009, IY001 and IY034 was over 72%, but that between the isolates of IY009 and IY008 was 30%.

To elucidate the correlation between the structural characteristics and the tumor immunological activity of GLG, the cultural broth of mycelial was fractionated as T-AS, UF-AS, M-AS, S and H fractions. And they were further fractionated as WS and WI fractions. The highest level of GLG was observed in H fraction of among these and UF-AS, T-AS fractions were obtained at the level of 2.32 g/l and 2.07 g/l, respectively.

T-AS was composed of 70.3% of carbohydrate and 7.8% of protein. T-AS obtained from *G. lucidum* was a heteroglucan composed of D-glucose, D-fructose, D-galactose, D-mannose, D-Xylose, and small amounts of D- arabinose and D-ribose. The WS fractions showed higher carbohydrate content than WI fractions, and glucose content of GLG fractions ranged from 63.3% to 92.2%. Also they were mainly composed of β-glucan as a result of gas liquid chromatography. Amino acid analysis showed that alkali treated GLG fractions contained a large amount of aspartic acid, glutamic acid, alanine and leucine, and the fractions not treated with alkali were highly composed of serine and threonine. These facts showed that sugar moiety of native polysaccharide was presumed to be linked with serine and threonine of protein.

T-AS had two polymer peaks, a higher molecular weight peak of 2,000 kD which represents about

24.5% of total glucan(as recovery mass), and a low molecular weight peak of 12 kD which accounts for about 75.5% of total glucan. T-AS showed characteristics I.R. adsorption for β-glucosides at 890cm^{-1} and ^{13}C-NMR spectroscopy confirmed the presence of the β-1,3-glucan and a β-1,6-glucan. T-AS was composed of 38.9% of C, 5.7% of H, 49.6% of O and 1.84% of N and the melting point of the polysaccahride was at 163°C.

The highest antitumor activity was observed in T-AS of the GLG fractions, but that the native glucan such as H and S fraction had little effect on the tumor. T-AS which has the antitumor activity of administration of intraperitoneal at a dose of 1.8 mg/kg reached the same level as that found with oral at a dose of 29.5 mg/kg.

T-AS can activate the complement activation via the alternative and the classical pathway in a dose-reponse manner, suggests that activation of the complement system may partially take part in immunotheraphy of tumors.

Changes of a specific serum proteins have recently received much attention on the studies of immunomodulators. The concentration of LA, LB and LC in the serum of normal ICR mice increased at the highest level on 4 days after final injection of T-AS, then gradually decreased. Also T-AS observed strong potentiators of hemolytic plaque-forming cell production of spleen cell in mice, it might possibly be due to the helper T-cell activation.

Above the results, the antitumor activity against sarcoma 180 bearing ICR mouse of GLG fractions of T-AS, UF-AS, M-AS, H and S were correlated with their immunological activities, such as anticomplementary activity, secretion of specific serum protein, production of hemolytic plaque-forming cell.

T-AS was enhanced the carbon clearance activity and the activity of T-AS was slightly inhibited by treatment of mice with a brocker of the macrophage function. These results suggested that the antitumor activity of T-AS obtained from *G. lucidum* IY009 might be mediated by the immunological potentiation involving macrophage.

Raw 264.7 and murine peritoneal macrophage treated with GLG or stimuli enhanced production of the nitric oxide, TNF-α and IL-1α.

It has been shown that NO by GLG was stimulated by induced TNF-α at an in early stage of macrophage activation. Also, these production by GLG had differnt from the chemical composition and structure properties according to the GLG fractions, expecially WI fraction of GLG exerted more favorable influence on NO production than the WS fraction did remarkably. Expecially, the highest production of nitric oxide and TNF-α was observed in T-AS-WI fraction. NO production by T-AS treatment was dependent on the concentration of TNF-α induced by GLG-stimulating macrophage, protein kinase activators and inhibitor had no effect on the production of NO and TNF-α. These results

suggests that the mode of NO and TNF-α induction by T-AS was independent on the PKC biological activities *in vitro*, and GLG to induce the TNF-α and IL-1 is functioned by a possible independent stimulation manner.

The culture supernatants of Raw 264.7 cell treated with GLG increased tumoricidal activity against sarcoma 180, P388, L929 and Yac-1. The cytotoxicity of sarcoma 180 and L929 cell was propotional to NO and TNF- concentration. Also the NO secretion and generation of macrophage-mediated tumoricidal activity were inhibited by L-NAME, a specific inhibitor of NOS.

When the effector cell and target cell were cocultured with T-AS and L-NAME, the cytotoxicity against sarcoma 180 and Yac-1 tumor cell lines were as dependent on the production of NO. Whereas, the presence or absence of L-NAME did not alter the cytotoxicity against L-1210, P388 and L-929.

T-AS, alkali extracted from the mycelia of *G. lucidum* IY009 stimulated the proliferation of splenocytes, bone marrow and thymocytes and was found to significantly enhance the release of murine IL-2 and IFN-λ.

The above results demonsrate that GLG contains immunoregulatory factors responsible for stimulating macrophage, which secretes NO, TNF-α and IL-1α playing an important role in immune response and suggest that the cytokines such as IL-2 and IFN-λ were important mediator of host deffence to tumor cells.

이 준 우

1995 건국대 대학원(박사, 생물공학 전공)

1987 건국대 대학원 졸업(석사, 발효생산전공)

1985 건국대 미생물공학과 졸업(학사)

1988. 6.~현재 일양약품(주) 중앙연구소 생물공학실 책임연구원

1987. 10~1988. 5 해태제과(주)

1985. 10~1987. 5 한국과학기술원 유전공학 센터 위촉연구원
