

## Antiseptic activity of ginsan with

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Oriental herbal medicines have been used for the treatment of various diseases for more than 2,000 years, and there is strong evidence that co-administration of herbal medicine with conventional therapy can reduce the side effects of these treatments or improve general conditions of patients, especially in

cancer and infectious disease. In recent decades, polysaccharides isolated from botanical sources (mushrooms, algae, lichens and higher plants) have also attracted a great deal of attention in the biomedical arena because of their broad spectrum of therapeutic properties and relatively low toxicity (1-3).

### What is ginsan?

**Jie-Young Song:** In the search for a new immunomodulator from natural sources, we screened 70 kinds of traditional medicines for their effect on proliferation of lymphocytes, generation of LAK cells and activation of macrophages. Among them, the most promising effect was observed in the water extract of ginseng. Ginseng is one of the most widely used medicinal plants throughout Far East Asian countries including China, Korea and Japan. Extensive investigations suggest the saponins in ginseng to be the key ingredients exhibiting the adaptogenic, anti-stress and antitumor activities. However, the saponins (including ethanol extracts of ginseng) did not show any immunostimulatory effects in our screening system. We further studied to purify the active component from water extract of ginseng, and elucidated polysaccharides named ginsan that was composed of  $\alpha(1\rightarrow6)$ -glucan and  $\beta(2\rightarrow6)$ -fructan in a 5:2 molar ratio and its average molecular weight is 2000 KDa.

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### How can ginsan induce resistance to experimental sepsis?

**Jie-Young Song:** Based on the recent results, ginsan modulates monocyte/macrophage-mediated innate immunity largely by up-regulating phagocytosis and enhancing bacterial clearance and down-regulating inflammatory cytokine synthesis. The reduced inflammatory responses are the consequence of decreased signals transmitted via TLRs, JNK, p38 MAP kinases, and NF- $\kappa$ B. Interestingly, the fact that ginsan alone stimulates TLRs in normal macrophages, but down-regulates them in septic macrophages, leads us to speculate that ginsan can induce tolerance against

a variety of septic challenges. In support of this hypothesis, endotoxin or LPS tolerance is a well-established phenomenon in which pre-exposure to a sublethal dose of LPS blunts subsequent lethal LPS-induced mortality, and this effect was closely associated with diminished production of proinflammatory cytokines (4,5). The distinguishable characteristic of ginsan may be exhibiting a wide spectrum of bacterial tolerance in Gram-positive, Gram-negative, and polymicrobial septic models and did not induce lethal shock, even at high concentrations.

### Can ginsan modulate macrophage function?

**Jie-Young Song:** Murine peritoneal macrophages on *in vitro* treatment with ginsan significantly induced production of cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and IL-12 as well as reactive nitrogen species. As these cytokines are directly involved in the process of macrophage activation and play a major role in resistance against infectious agents and tumor cells, ginsan consequently enhancing macrophage-mediated cytotoxicity in a dose-dependent manner. Co-incubation of ginsan activated macrophages with tumor cells either up- or down-regulated the production of different cytolytic molecules depending on tumor

cells, thereby modulating susceptibility of these tumor cells to macrophage-mediated tumor cytotoxicity. Ginsan treatment also increased the background level expression of MHC class II molecules that is a principal mechanism of enhancing the interaction between macrophages and T cells, suggesting that ginsan stimulates macrophages to activate other immune cells via cell-to-cell communication resulting in the modulation of various immune responses. In contrast, the expression of CD11b is decreased in ginsan treated macrophages, indicating that ginsan may not affect the migration of macrophages.

### Has ginsan any effects on cytokine secretion or TLR-expression?

**Jie-Young Song:** Since ginsan has a potent immunostimulatory effect, ginsan increased mRNA expressions of a variety of cytokines (Th1, Th2 and proinflammatory cytokines) in murine splenocytes *in vitro*. Interestingly, ginsan treatment significantly decreased the proinflammatory cytokines in septic mice, including TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IFN- $\gamma$ , IL-12, and IL-18, whereas Th-2 type cytokines (IL-2 and IL-4) were not affected. On the contrary, ginsan increased the above cytokines in irradiated mice, which were shifted to Th2 polarization. Thus, we propose that ginsan treatment eventually restored the balance between the pro- and

anti-inflammatory arms (Th1 vs. Th2) of the cytokine network in disease-associated state, thereby maintaining homeostasis.

As mentioned above, ginsan itself increased some TLRs in spleen. Nevertheless, ginsan pretreatment dramatically suppressed the expression of augmented TLR2, TLR4, and TLR9 in *S. aureus*-infected macrophages, ultimately leading to a reduction in inflammatory cytokines. We assume that pretreatment of ginsan could induce an adaptive response showing resistance to second stimulation of bacteria via down-regulation of TLR or inhibition of signaling cascades in multiple ways.

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