5. Summary

Ganoderma lucidum, a traditional Chinese medicine, harbors a well-known capacity to modulate immunoactivity and inhibit tumour cell growth. GLIS is a proteoglycan fraction purified from *Ganoderma lucidum* through different chromatographic steps. This fraction has been proven to induce the proliferation and differentiation of lymphocytes. Its effect on B cells and macrophages was further investigated.

GLIS was also found to be the active fraction of crude extract *Ganoderma lucidum* in the activation of macrophages. After being stimulated with GLIS, the cells were more spread out and elongated than those of controls. It can activate macrophages to increase the secretion of IL-1β, TNF-α and reactive nitrogen intermediate (RNI) production. The percentage of phagocytosis was significantly enhanced in the presence of GLIS, and it triggered macrophage activation for tumour cytotoxicity. The macrophages from tumour-bearing mice demonstrated more sensitivity to the stimulus of GLIS.

GLIS selectively increased the proliferation of B cells, and enhanced the population of CD25 and CD71 positive cells of B cells, but not that of non-B cells, so the cell-type specificity of GLIS was B cells. After being stimulated by GLIS, B cells were activated into antibody producing plasma cells, and secreted significant amounts of IgM. With the respect to the response to MSLs, the relative amount of IgM obtained from B cells induced by GLIS was lower in the absence of accessory cells. When 0.5% macrophages was added to B cells, B cell survival and IgM secretion increased significantly after stimulation with GLIS. The interaction of B cells and macrophages was not only due to the substances secreted by macrophages, but also to the direct effect. The B cells from tumour-bearing mice could secrete 3 - 4 times IgM than that of TMSLs. *In vivo* experiments also found a significant increase of IgM secretion after injection with GLIS in comparison to the control.

Characterisation of the active fraction GLIS was performed. Polymyxin B, a selective inhibitor of LPS, abolished LPS-induced NO production of macrophages, whereas it did not inhibit the action of GLIS on macrophages. Through HPLC, the active compound of GLIS was determined with the molecular weight ca. 2000 k. The activity of GLIS did not change after treatment with pronase E, but the activity was significantly reduced after treatment with $NaIO_4$. When GLIS was digested with β-1,3-glucanase, the activation rate of macrophage was reduced significantly.

FITC-labeled GLIS can bind to macrophage surfaces as confirmed using FACS analysis. Incubation of macrophages in the presence of either laminarin or mannose, which are soluble carbohydrate antagonists of macrophage β-glucan and mannose receptors, significantly reduced macrophage NO production following treatment with GLIS. Treatment of macrophages with anti-CD14 Ab significantly blocked GLIS-induced nitrite production.

According to their traditional use in China, eight traditional Chinese medicines were chosen to be screened for their inhibition activity of tumour cells. *Polygonum cuspidatum* demonstrated strong tumour growth inhibition. The active compounds of the crude extract

were sought through bioassay-directed fractionation. HZ-3-1-b was shown to be the active compound in the crude extract of *Polygonum cuspidatum* that inhibited the growth of tumour cells.

HZ-3-1-b could inhibit the proliferation of various kinds of tumour cells in a dose-dependent manner. HZ-3-1-b was confirmed to induce SW 620 cells apoptosis by stained with annexin V-FITC conjugate and analysis of DNA fragments. In addition, it was found that SW620 cells were arrested in S phase. HZ-3-1-b was identified as resveratrol using HPLC and TLC.

Through resveratrol affinity column, a protein with a molecular weight of ca. 35-40 k was found. It was identified as GAPDH through Maldi-TOF analysis. Reveratrol could inhibit the activity of GAPDH. V_{max} of the enzyme was down to 21% by 100 μm reveratrol, whereas K_m remained unchanged.