

Effects of plant lectins on gene expression in animal cultured cells and living tissues

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Lectins are proteins or glycoproteins of non-immune origin that bind specifically to the carbohydrate moiety of glycoconjugates. They have various biological activities, causing cell agglutination, mitosis, toxicity and cell growth inhibition. Mistletoe lectins also have various biological activities including anti-cancer and immunomodulatory effects. To examine the effects of ML-J on cytokine gene expression in human colonic carcinoma Caco-2 cells and in the mouse intestine, mRNA expression levels of different cytokines were measured by reverse transcription-polymerase chain reaction and quantitative real-time polymerase chain reaction.

Japanese mistletoe lectin (ML-J) was purified by affinity chromatography and gel filtration. The purity of ML-J was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Caco-2 cells were cultured and incubated with ML-J for different time and concentration. Groups of mice were intragastrically given lectins (10 µg/head) and the duodenum, ileum, and Peyer's patch were removed. Total RNA was extracted from the cells and tissues, and cDNA was prepared. Gene expression of the proinflammatory cytokines interleukin (IL)-8, tumor necrosis factor- α (TNF- α), and IL-6 in Caco-2 cells and TNF- α and IL-6 in the tissues were measured.

The results of SDS-PAGE revealed that ML-J was more than 95% pure. RT-PCR showed that when Caco-2 cells were treated with ML-J at 10 ng/ml, the gene expression of TNF- α , and IL-8 increased time-dependently and when cells were incubated with ML-J for 24 h, the gene expression of IL-8, TNF- α and IL-6 increased concentration-dependently. These results were confirmed by Q-PCR. In the mouse duodenum, ML-J caused an up-regulation of the gene expression of TNF- α and IL-6 as revealed by RT-PCR and Q-PCR. There were no alteration in the expression of these genes in the ileum and Peyer's patches. The results of RT-PCR and Q-PCR clearly demonstrated that ML-J up-regulated the gene expression of IL-8, TNF- α , IL-6 in Caco-2 cells and TNF- α and IL-6 in the mouse duodenum. In summary, the present results indicated that ML-J exhibited strong stimulatory activity to enhance the gene expression of certain proinflammatory cytokines *in vitro* and *in vivo*.