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Partial Purification of Mitogenic Material and Study of Tumoricidal Activity of *Duchesnea chrysantha*

Teahun Kim* and Gajin Jeong
School of Biological Science, Seoul National University. Seoul 151-742

Duchesnea chrysantha has mitogenic activity on murine immune cells. Mitogenic material has been extracted through blending with 5% ethanol. The crude extract 70% ammonium sulfate and partially purified through DEAE-cellulose anion exchange chromatography, gel Electro elution on denaturation condition and Con- A affinity chromatography. The molecular weight of mitogenic material was about 17KDa as determined by sodium dodecyl sulfate gel electrophoresis. ISL-II, a partial purified mitogenic material, induces mitosis and blastogenesis at murine splenic lymphocyte cells. This phenomenon is morphologically similar to LPS- induced mitosis on microscopy. In the study of survival rate of tumor bearing Babl/c mouse. Intraperitoneal injection of a partially purified mitogenic material resulted in suppressed tumor growth in tumor bearing Babl/c mouse and 40% of mouse have even completely cured. In contrast a partially purified material not treated all of tumor bearing mice have died. In the study of RAW264.7 activation treated with ISL-II in vitro, it enhanced nitric oxide production in RAW 264.7 five folds. A mitogenic material agglutinated SRBC. In that point we suggested that this material had glycosidic residue. For that reason mitogenic material had tumoricidal, mitogenic activity. We also confirmed the suggestion through elution of a mitogenic material by Con-A affinity chromatography. These results imply that ISL-II, partially purified mitogenic material, has the activity of enhancing lymphocyte mitosis, macrophage activation and tumoricidal effect.

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Antitumor and Immune Enhancing Effects of Lactic Acid Bacteria Isolated from Kimchi

Jungeun Lee* and Gajin Jeong
School of Biologocal Sciences, Seoul National University, Seoul 151-742

Many investigators have studied the therapeutic and preventive effect of lactic acid bacteria on disease such as cancer, infection, gastrointestinal disorders, and asthma. Because the immune system is an important contributor to all of these diseases, an immunostimulatory effect of lactic acid bacteria has been investigated by using mainly animal models. We isolated lactic acid bacteria (LAB) from kimchi, korean traditional fermentated food. The LAB from kimchi was identified lactobacillus (Lac1-2) and Leconostoc (IH22) by several biochemical test. Antitumor activity of Lac1-2 and IH22 was studied in BALB/c mice by using Sarcoma 180 *in vivo*. Intraperitoneal injection with Lac1-2 or IH22 prolonged the life span of mice inoculated i.p with Sacorma 180 cells compared to that of control mice. The antitumor activity of Lac1-2 and IH22 was reduced when injected i.p with carrageenan, an anti-macrophage agent. And we tested the enhancing effects of Lac1-2 and IH22 on macrophage activation *ex vivo*. Intraperitoneal injection of Lac1-2 or IH22 increased NO and H_2O_2 production of peritoneal cells of mice. In the RAW264.7, a macrophage-like cell line, NO and H_2O_2 production was increased *in vitro*. In the experiment for TNF Bioassay, treatment with culture supernatant of RAW264.7 was inhibited proliferation of L929 cell line. Also, oral administration of LAB from kimchi prolonged the life span of mice infected with *Salmonella typhimurium* compared to that of control mice. This result shows that LAB

from kimchi plays an important role in the prevention of enteric infections. And the proliferative responses of spleen cells to concanavalin A (a T-cell mitogen) and lipopolysaccharide (a B-cell mitogen) were also siginificantly enhanced in mice feeding LAB from kimchi. So, we proposed that antitumor activity of LAB from kimchi is through macrophage activation and oral administration of LAB from kimchi enhanced immune system.

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Induction of apoptosis of human monocytes by human cytomegalovirus

Myung Sook Moon[*1], Yeon-Hee Cho[*1] and Chan H. Lee[*1,2]

Division of Life Sciences[1], and Research Institute for Genetic Engineering[2], Chungbuk National University, Cheongju, Chungbuk 361-763

Effect of human cytomegalovirus (HCMV) on three human monocyte cell lines at different stages of differentiation was investigated. While the viability of HL60 cells or U937 cells was not significantly affected by HCMV infection, the viability of THP-1 cells was reduced. Acridine orange/ethidium bromide staining revealed that the reduction of THP-1 cell viability was due to increased apoptotic death following HCMV infection. Apoptosis of HL60 cells was not affected by HCMV infection, and induction of apoptosis of U937 cells by HCMV was intermediate of HL60 and THP-1 cells. Since HL60 cells are the least differentiated and THP-1 cells are the most differentiated the induction of apoptosis of human monocytes appears to be related with the degree of cell differentiation. Induction of apoptosis of THP-1 cells by HCMV did not require viral gene expression, since UV-inactivated HCMV also induced THP-1 cell apoptosis. Physical contact of HCMV virion particles with THP-1 cells seemed to be required for apoptosis because apoptosis was reversed by treating virus-infected cells with heparin, preincubating virus particles with trypsin or THP-1 cells with heparinase. Fluorescence and confocal microscopy using fluorescent calcium indicator Fluo-3 suggested an increase in cytosolic calcium concentration in THP-1 cells undergoing apoptosis. Calcium influx blcokers such as verapamil and nifedipine partially reversed HCMV-induced apoptosis of THP-1 cells.

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Interaction of Human Cytomegalovirus Particles with Heparan Sulfate on the Cell Surface Stimulates Human Leukocyte Antigen Class I Expression

Byung H. Song[*1], Gyu C. Lee[*1] and Chan H. Lee[*1,2]

Division of Life Sciences[1], and Research Institute for Genetic Engineering[2], Chungbuk National University, Cheongju, Chungbuk 361-763

Human cytomegalovirus (HCMV) is known to down-regulate the expression of HLA class I, of which process involves a subset of viral genes. Infection of human fibroblast cells with UV-inactivated HCMV (UV-HCMV), however, resulted in an enhancement of HLA class I expression. Heparin, which can interfere with interaction of viral particles with heparan sulfate proteoglycans on the cell surface, completely blocked the effect of UV-HCMV on HLA class I expression. Treatment of cells with heparinase or UV-HCMV with trypsin decreased in a dose-dependent manner the effect of UV-HCMV on enhancing HLA class I expression. Sodium chlorate, which is known to inhibit the sulfatation of heparan sulfate proteoglycans, gave a similar result. Thus, binding of HCMV particles to heparan sulfate proteoglycans on the cell surface appears to be involved in enhancement of HLA class I expression.