

D017

Protein Profiling of Anthrax-intoxicated Murine Macrophages Using SELDI-TOF

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Anthrax is caused by *Bacillus anthracis*, a gram-positive, spore-forming, rod-shaped, toxin-produced bacterium. Anthrax lethal toxin (LeTx) consists of protective antigen and lethal factor. LeTx is a zinc-protease that cleaves certain MAP kinase kinases, leading to death of the host via a poorly defined sequence of events. Here, we have used SELDI-TOF-MS to discriminate between the different expressed proteins from anthrax LeTx-intoxicated RAW 264.7 cells. The method is based on the differential binding of protein subsets to chemically modified surfaces. Cytosolic fractions of intoxicated macrophages were added to anionic affinity SELDI chip surfaces. After binding, washing, and SELDI-TOF-MS different protein profiles were obtained. Among twenty different protein peaks, the 7,520 Da was decreased specifically in LeTx intoxicated cells at 60 min and the 3,973 Da was increased at 90 min and 180 min. These protein peaks imply that cell death due to the LeTx can be occurred through complex pathways. Thus, the protein profiling approach based on SELDI-TOF-MS holds great promise for rapid high-resolution identification of intoxicated cells.

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D019

Anti-fungal Activity and Anti-inflammatory Effects in Murine System of *Streptomyces* sp. SUS-0608 Isolated from Soil

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Of them active compound from *Streptomyces* sp. SUS-0608 was found to be an anti-fungal agent (AFA) and showed immunosuppressive activity on mouse splenocytes, mitogen stimulated splenocytes, macrophages viability and proliferation *in vitro* and *in vivo*. As an immediate host defense reaction, the innate immune response involves secretion of cytokines and other mediators leading to an inflammatory process that has antimicrobial effects. One important and widely-used transplantation factor that plays a pivotal role in many cellular responses is NF- κ B. NF- κ B is a DNA-binding protein that is important for maximal expression of many genes that are involved in inflammatory responses. As a result, production of NF- κ B-mediated cytokine and radical that is important in inflammation is reduced to suppress immune response by AFA when macrophages are incubated with AFA for short culture time. AFA also induces apoptosis that is important in homeostasis in macrophages and splenocytes when cells are incubated with AFA for long culture time. The subcutaneous air-pouch has been found to be suitable for studying chronic granulomatous inflammation. And for the study related to rheumatic disease, it has the closest approximation to synovial tissue because it is only other blind connective tissue cavity which lacks a mesothelial basement membrane. The similarity of synovium was found to be closest for 6 days after air infection. In this study, air-pouch was made on the back of ICR mouse, and stimulated with carrageenan. And inflammatory processes were detected with the exudates.

D018

Effects of Concanavalin A on Human Peripheral Blood T cell Apoptosis and Cytokine Production.

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Concanavalin A (Con A), normally a mitogen of T-lymphocytes (1 ~ 10 μ g/ml), is an apoptosis-inducing agent of T-lymphocytes when treated with high concentration (> 30 μ g/ml). In the process of Con A-driven apoptosis on T-lymphocytes, many cytokines were secreted into extra-cellular space promoting B cell terminal differentiation.

In this research, the progress of apoptosis was measured by flow cytometry, gel electrophoresis. Cytokine expression was estimated by RT-PCR and ELISA and antibody production was estimated by ELISA (Enzyme-Linked Immunosorbent Assay) on SKW 6.4. Expression of I-kappa B was measured by immunoblotting.

This research suggests that Con A induces many cytokines on early phase of T cell apoptosis at the high concentration. Cytokines were expressed through NF-kappa B and Cytokines of the early phase finally induce antibody production of B-lymphocyte.

D020

Immune Stimulatory Effect of Partially Purified Compound from *Streptomyces* sp. CGS-1015

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Many secondary metabolites from *Streptomyces* spp. have immunomodulatory activities and antifungal activities. In this study, we tested immunomodulatory activities in culture broth of *Streptomyces* sp. CGS-1015 that were partially purified by DEAE-Sephacel ion exchange chromatography, and characterized it. The bioactive compound was partially purified in 0.4M NaCl by DEAE-Sephacel ion-exchange chromatography. And we have investigated the effect of these partial purified compounds (C-IE) on proliferation of mouse splenocytes, activation of mouse macrophage and expression of inflammatory mediator-proinflammatory cytokine, nitric oxide and transcription factor. We found that C-IE enhanced the activation of mouse macrophage - Raw 264.7 and peritoneal macrophage - by increasing of production of NO, expression of inflammatory cytokine and enhanced phagocytosis. Also the expression of these inflammatory mediators were controlled by NF-kB and C-IE controlled the expression of this transcription factor. And we identified CGS-1015 sp. These results imply that C-IE, partially purified immune stimulatory molecule, has the activity of enhancing lymphocyte mitosis, macrophage activation. And also we expect this molecule would have tumoricidal and antifungal effects.