

[PB4-20] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Immunostimulatory Effect of Ginkgolides Enhances Resistance of Neutropenic Mice against Hematogenously Disseminated Candidiasis.

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We investigated immunoactivity of ginkgolides (GLS), the primary active constituent of Ginkgo biloba leaves, against disseminated candidiasis due to *Candida albicans*. This fungus is a polymorphic opportunistic pathogen. BALB/c mice were induced neutropenia by intraperitoneal (i.p.) injection of cyclophosphamide (CP) 24 hours before an i.p administration of GLS (2 mg/mouse) to the mice. Control mice received diluent (Dulbecco's phosphate saline solution; DPBS) instead of GLS. Four hours later, all of these mice were infected with live *C. albicans* yeast cells (2×10^5 /mouse), intravenously. Prior to the experiments, an appropriate amount of CP that caused depletion of neutrophils in BALB/c mice was examined and found as 2 mg/mouse. In addition, antifungal activity of GLS were determined by susceptibility test. The GLS analyzed in our other study were used. Results from measurement of survivability showed that GLS-treated mice had a mean survival time of 36 days, compared with 14 days for DPBS-given control mice. Three mice of the five GLS-treated animals survived the 52-day observation period. The antifungal susceptibility test resulted in no killing of the yeast at a concentration of 50 mg GLS, showing that the GLS had no antifungal activity. Our studies show that the ginkgolides exhibit immunostimulatory effect.

[PB4-21] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Dendritic Cell as an effective cancer immuno-cell therapy module II. : Anti-tumor effect of cultured DCs in murine melanoma metastasis model

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Dendritic cells (DCs) are known to professional antigen presenting cell (APC). Due to the role as an effective activator of cytotoxic T Lymphocytes by expressing MHC, adhesion and co-stimulatory molecules, DCs are now widely recognized to play an important role in the immune responses to tumors. We investigated the effect of cultured DCs in murine melanoma pulmonary metastasis model. To follow the metastasis protocol, syngenic melanoma cells were inoculated intra-venously into the mouse (B16F10 into the C57BL/6) 8 days prior to the first DC injection (1×10^6 DCs/ mouse, i.p.) and the autologous tumor cell lysate pulsed-DCs were injected as a therapeutic module twice in two weeks. Myeloid lineage cells were selected by antibody panning from mouse bone marrow cells. And ex vivo culture was done with GM-CSF and IL-4 (1,000 U/ml each) for 7 days in media. On the 6th day, DCs were pulsed with autologous tumor cell lysate for 18 hr (p-DC). One week after the final injection, mice were sacrificed for the systemic immune monitoring. Ex vivo cultured myeloid-DCs produced IL-12 more than IL-10 indicating the possibility of stimulating TH1-related immunity. In the auto-MLR assay, T cell proliferations (divisions) significantly increased by the DC stimulation. In the p-DC treated mice, increased antigen-specific IFN- γ production and the T cell proliferations were observed with the inhibition of pulmonary tumor nodule formation. These data indicating the effectiveness of cultured DCs to treat the pulmonary metastasized melanoma in mice.

[PB4-22] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Isolation of Ginkgolides and the Effect of These Components on Inflammation in Mice Induced by Complete Freund's Adjuvant.

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The major active components of EGb 761, extract of Ginkgo biloba leaves, include flavonoid glycosides and unique diterpenes known as ginkgolides. Ginkgolides are potent inhibitors of platelet activating factor. In this study, we investigated antiinflammatory activity of ginkgolides on the Complete Freund's Adjuvant (CFA)-induced mice. The ginkgolides were extracted from commercially available EGb 761. This extracting procedure was done by sequential treatments of the EGb 761 with chloroform, methanol, and water. HPLC and thin layer chromatography (TLC) analyses of the final water-soluble component (GH 415) revealed presence of the ginkgolides A, B, C, and J. For induction of arthritic inflammation, BALB/c mice were given CFA (50 μ l/mouse/injection) into their footpads at days 2, 3, 4, and 5, respectively. The mice were treated with GH 415 (2 mg/mouse/injection) before and after CFA-administrations intraperitoneally at an interval of 3 days such as days 1, 4, 7, 10, 13, 16, and 19. Control mice group received Dulbecco's phosphate saline (DPBS) instead of GH 415. Degrees of footpad swelling of these animals were then measured with plethysmometer. Results showed that the footpad swellings from all GH 415-treated mice were reduced up to 55% as compared to swellings from the DPBS-given control mice. This phenomenon of the reduction was maintained for the 33 day-measurement period as degrees of the footpad swellings all declined with or without the GH 415-treatment. These data indicate that the constituent of ginkgolides A, B, C, and J helps mice reduce inflammation.

[PB4-23] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Ginsenoside Rg3 inhibits the production of interleukin-1 β , tumor necrosis factor- α , and nitric oxide in rat microglia

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Inflammatory responses from activated microglia are one of major causes of Alzheimer's disease (AD). Particularly, proinflammatory cytokines (PC), such as IL-1 β and TNF- α , and nitric oxide (NO) are correlated with AD by inducing the chronic inflammation in the brain. In the present study, we found that microglia are activated by lipopolisaccharide (LPS) and Abeta42 (A β 42), and those activated microglia produced such repertoires up to 72h with a turning point at 24h. However, no dose dependency was found during the chasing time courses (6h to 72h). 100 μ g/ml of Rg3 showed the most effective result in all study tools, Griess reagent, RT-PCR, and EIA assay. In conclusion, the fact that Rg3 downregulates the release of such proinflammatory repertoire suggests that the brain cell can be protected from cell stresses caused by PC and NO and from the cell damage arisen from the chronic inflammation.

[PB4-24] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Enhanced apoptosis of IFN- γ treated macrophage in a depleted nutritional state

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Apoptosis has been implicated as an important mediator in immunosuppression observed in a depleted nutritional state. The recent report has indicated that IFN- γ treated bone marrow macrophages were protected from apoptosis induced by several stimuli in complete medium condition. However, our previous study demonstrated that IFN- γ treated peritoneal macrophages were enhanced the apoptosis in a depleted nutritional state. Therefore, we investigated the apoptotic regulatory mechanism of IFN- γ in malnutrition-induced macrophage. After peritoneal macrophages were isolated from C57BL/6 mice, purified macrophages were treated with IFN- γ in complete medium condition. The cells were further incubated in conditional medium condition. Apoptotic cells were determined by MTT assay, caspase-3 assay, PI staining and DNA fragmentation assay. Apoptotic cells of IFN- γ treated macrophages were increased as compared with those of untreated macrophage. Moreover, Caspase-3 activity and Bax expression in IFN- γ treated macrophages was increased, whereas Bcl-xL expression was decreased. Apoptosis of IFN- γ treated macrophages was not induced in complete medium condition. These data