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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 μl/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-1β, 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-1β 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 (Aβ42), 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㎍/㎖ 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. Therefor, 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

demonstrate that IFN-γ enhances apoptosis in malnutrition-induced macrophages, suggesting that apoptotic regulatory mechanism of IFN-γ in malnutrition-induced macrophage is different from complete medium condition.

[PB4-25] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Gamma-irradiation induced expression of ICAM-1 on human meuroblastoma cells is mediated by the activation of p38 MAP kinase.

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Since radiotherapy has been suspected to promote tumor metastasis and the presence of increase levels of adhesion molecules have implications for metastasis, we decided to investigate whether gamma-irradiation alters the expression of intercellular adhesion molecule-1 (ICAM-1) on neuroblastoma cells and the activities of relevant intracellular signaling molecules. In the present study, the relative of ICAM-1 expression under gamma-irradiated neuroblastoma cells were assessed by Western blot analysis. Our data indicated that gamma-irradiated neuroblastoma cells significantly increased the ICAM-1 protein level in a dose dependent manner. Also, we showed that treatment of neroblastoma cells with gamma-irradiation resulted in increase NO release. The effect of gamma-irradiation on activation of NF-kB transcription factor was determined by Western Blotting and our data showed that NF-kB is not involved by gamma-irradiation. We further investigated the effect of PKC, p38 and MEK inhibitors on radiation-induced expression of ICAM-1 by Western Blotting and demonstrated that ICAM-1 expression was partially blocked by p38 inhibitor. We also estimated the NO production in these inhibitors-treated groups and showed that p38 inhibitor significantly increased the NO production in gamma-irradiated cells. These results suggest that NF-kB transcription factor is not involved in gamma-irradiated ICAM-1 expression and the NO production and ICAM-1 expression by gamma-irradiation may be mediated through p38 kinase pathway in neuroblastoma cells.

[PB4-26] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Release of the Pro-inflammatory Cytokines and Facilitation of Immune Response in LPS-induced Activation of Macrophage by Crude Cordycepin Containing Adenosine (CCCA) from Cordyceps militaris

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The in vitro effects of extracted fractions of C. militaris on the secretion of cytokines in murine macrophage cell line, RAW 264.7 were studied. F1 (crude cordycepin containing adenosine), F2 (ethanol precipitation), F3 (ethanol soluble supernatant) and F4 (fraction of through SK-1B) significantly stimulated the production of cytokine and nitric oxide (NO) on murine macrophage cell line RAW264.7. We examined how the ethanol extract of C. militaris regulates production of interleukine 1-beta(IL-1β), tumor necrosis factor-alpha (TNF-α), and NO in vitro. F1 (5 μg/ml) and F4 (5 μg/ml) inhibit these inflammatory mediators in lipopolyisaccaride (LPS)-stimulated murine macrophage cell line RAW264.7 by suppressing protein expression of IL-1β, TNF-α, inducible nitric oxide synthase, and cyclooxygenase-2. Moreover, the extract suppresses the nuclear transcription factor (NF-κB)-kappa B activation in LPS-stimulated RAW264.7 cells. However, the production of the macrophage cytokines, IL-1 and TNF-α, by RAW 264.7 treated with F3 was examined from 20 up to 40 μg/ml with dose dependent manner. NO production was also increased when cells were exposed to combination of LPS and F3 from 1.2 up to 40 μg/ml. These results indicate that the C. militaris ethanol extract suppresses inflammation through suppression of NF-κB-dependent inflammatory protein expression, suggesting that the C. militaris extract may be beneficial for treatment of endotoxin shock or sepsis.