inflammatory activities in several animal models and in a clinical study on patients with OA, JOINS was revealed to have a good analgesic efficacy and safety profile.
In this study, we tried to evaluate the possibility of JOINS as a curative therapeutics of rheumatoid arthritis using several in vitro and in vivo models. JOINS inhibited adjuvant-induced arthritis in rats and reduced inflammatory pouch volume and capillary permeability. JOINS also attenuated the PMA-stimulated chemilunminescence in neutrophils and degradation of articular cartilage by oxygen radicals. JOINS decreased conA-stimulated T cell proliferation and LPS-stimulated B cell proliferation. In conclusion, JOINS has a strong potentiality to be developed as a safe drug for rheumatoid arthritis.

[PA1-61] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Mucogen Ameliorates the Fibrosis and Inflammation of Chronic Pancreatitis in Mice

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In the present study, we established experimental chronic pancreatitis model in mice through repetitive induction of acute pancreatitis with intraperitoneal injections of cerulein (40 mcg/kg, 6 times every hours twice per week for 5 or 10 weeks), which led to chronic pancreatitis with fibrosis. Severe pancreatic acinar atrophy, trans-differentiation of acinar to duct like tubular complexes, islets hyperplasia, and dilatation of intraacinar lumina developed. Masson-Trichrome staining demonstrated progressive accumulation of extracellular matrix in interlobar and interacinar spaces. The extents of pancreatic fibrosis were statistically significantly decreased in accordance with lessened pancreatic inflammations after treatment of Mucogen (DA-9601), phytopharmaceutical showing antioxidative and cytoprotective actions. Using nuclear extracts from pancreas and radiolabeled NF-kappaB probe, EMSA was done, which showed the increased NF-kappaB binding in chronic pancretitis and significantly attenuated NF-kappaB binding activities after mucogen treatment. The levels of myeloperoxidase and iNOS activities were also significantly decreased in mucogen treated group compared to pancreatitis control group. Cytoprotective proteins such as heat shock protein-70 and metalotheionine were siginificantly increased in mucogen-treated group. Mucogen decreased the expressions of alpha-SMA and type I collagen in cultured pancreatic stellate cells. Conclusively, we could establish the mouse model of chronic pancreatitis and mucogen might be considered as therapeutics in the prevention and treatment of chronic pancreatitis with fibrosis.

[PA1-62] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

G protein-coupled phosphoinositide 3-kinase γ is required for autotaxin-mediated tumor cell motility

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Cell motility is a fundamental process required during normal embryonic development, inflammatory responses, wound healing, and tumor metastasis. Autotaxin (ATX) is a 125-kDa glycoprotein secreted by the human melanoma cell line A2058. This autocrine motility factor has been shown to stimulate random and directed motility of human tumor cells at high picomolar to low nanomolar concentrations (ED50 = ~300-500 pM). In the present study, we have shown that G protein-coupled phosphoinositide 3-kinase (PI-3 kinase) γis involved in the signal transduction of Autotaxin (ATX), a novel tumor cell motility-stimulating factor. Pretreatment of the cells with PI-3 kinase inhibitors, wortmannin or LY294002 inhibits ATX-induced motility. Reverse transcriptase PCR and Western blot analysis showed that human melanoma cells have PI-3 kinase, p110γ. ATX increased the PI3-kinase activity in p110γ, but not p85, immunoprecipitates, which can be abolished by pretreatment of PI-3 kinase inhibitors (wortmannin, LY294002) or pertussis toxin. Collectively these results strongly suggest that PI-3 kinase p110γ is

involved in ATX-mediated tumor cell motility stimulation.

[PA1-63] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Delayed ERK Activation by Ceramide Reduces Melanin Synthesis in Human Melanocytes

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We investigated the effects of sphingolipid metabolites and the possible signaling pathways involved in human melanocytes. Our data shows that C2-ceramide inhibits cell growth in a dose-dependent manner, whereas SPP has no effect. Moreover, we observed that the melanin content of the cells was significantly decreased by C2-ceramide. The pigmentation-inhibiting effect of C2-ceramide at 1-10 μM was much stronger than that of kojic acid, tested at 1-100 μM. The tyrosinase activity of cell extracts was reduced by C2-ceramide treatment. However, in the cell-free system, C2-ceramide could not suppress tyrosinase, whereas kojic acid directly inhibited tyrosinase. These results suggest that C2-ceramide decreases the pigmentation of melanocytes indirectly regulating tyrosinase. Furthermore, we found that C2-ceramide inhibited the production of microphthalmia-associated transcription factor (MITF), which is required for tyrosinase expression. To identify the signaling pathway of ceramide, we studied the ability of C2-ceramide to influence extracellular signal-regulated protein kinase (ERK) and Akt/protein kinase B (PKB) activation. C2-ceramide induced a delayed activation of ERK (>1 h) and a much later activation of Akt/PKB (>3 h) in human melanocytes. In addition, the specific inhibition of the ERK and the Akt signaling pathways by PD98059 and LY294002, respectively, increased melanin synthesis. Thus, it seems that sustained ERK and Akt activation may lead to the suppression of cell growth and melanogenesis.

[PA1-64] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Intracellular signaling pathway associated with sphingosylphosphorylcholine-induced contraction in feline ileal smooth muscle cells.

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It is proposed that SPC-induced contraction mechanism involves a certain G protein, phospholipase and kinases-dependent pathway. G protein subtypes of Gi1, Gi3 and Go are immunoblotted in feline ileum. To investigate which subtype mediates the contraction, permeabilized cells were used. Gi3 antibody-treated cells showed the decrease of contraction induced by SPC. In addition, incubation of [^{35}S]GTPγS with membrane fraction increased its binding to Gi3 subtype after SPC treatment, suggesting that the signaling pathways invoked by SPC were mediated by Gi3 protein. After treatment of PLC inhibitor neomycin, the contraction was reduced. Of existing PLC subtypes, PLCγ1 antibodies decreased SPC-induced response when treated to permeabilized cells. MEK inhibitor PD98059 and PKC inhibitor chelerythrine blocked the contraction significantly, but p38 MAP kinase inhibitor SB202190 did not. However, co-treatment of PD98059 and chelerythrine showed no significant difference. SPC-induced contraction was inhibited with the incubation of PKCε antibodies to penetrate permeabilized cells. Phosphorylation and activity of p44/42 MAP kinase and was increased by SPC treatment, which was reversed by pretreatment of inhibitors of signaling molecules that decreased SPC-induced contraction previously. Thus, in feline ileal smooth muscle cells, the mechanism of contraction by SPC involves Gi3 protein, followed by the activation of PLCγ1, PKCε, and p44/42 MAP kinase.

[PA1-65] [10/18/2001 (Thr) 14:00 - 17:00 / Hall D]

Autotaxin increase the motility is breast cancer cells