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Interleukin-12 (IL-12) plays a prominent role in the development of Th1 cell-mediated immune responses. Th1 cell-mediated immune responses have been implicated in the pathogenesis of chronic inflammatory autoimmune diseases. Thus, pharmacological control of IL-12 production may be a key therapeutic strategy for modulating immunological diseases dominated by Th1-derived cytokine responses. In this study we investigated the effects of costunolide, a prominent sesquiterpene lactone of Saussureae radix, on IL-12 production in mouse macrophages stimulated with lipopolysaccharide. Costunolide potently inhibited the production of IL-12 in a dose-dependent manner. The effect of costunolide on IL-12 p40 promoter activation was analyzed by transfecting RAW264.7 monocytic cells with p40 promoter/reporter constructs. The repressive effect mapped to a region in the p40 promoter containing a binding site for nuclear factor kappaB (p40-kappaB). Furthermore, activation of macrophages by lipopolysaccharide resulted in markedly enhanced binding activity to the kappaB site, which significantly decreased upon addition of costunolide. These results suggest that costunolide inhibited-inhibition of IL-12 production in macrophages may explain some of the biological effects of costunolide including its anti-inflammatory activity.

[PB4-12] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Possible receptors for bovine lactoferrin on human monocytic THP-1 cells

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A variety of biological functions of human LF (H-LF) and bovine LF (B-LF) have been demonstrated in host defense, especially in immune responses, antibacterial activity and transcriptional activation of cells. Here, we discuss the characteristics of the binding of B-LF to THP-1 cells and partly compare the binding patterns of B-LF and H-LF. When we examined the binding of B-LF to THP-1 cells by Western blot analysis using B-LF labeled with biotin and a lysate of THP-1 cells, we found that B-LF binds to at least three types of molecules with molecular weights of about 25, 35 and 65 kDa in THP-1 cells. Also, the binding of B-LF to THP-1 cells was strictly dose-dependent. Further analysis revealed that the molecules with a M.W. of about 35 kDa among those that bound to B-LF are associated with the surface membrane of THP-1 cells, whereas the other molecules were derived from the cytosolic fraction. Inhibition tests using various saccharides showed that the binding was not inhibited by addition of a high concentration (100 mM) of GlcNAc, GalNAc, mannose or lactose, suggesting that oligosaccharides attached to B-LF are not involved in the binding of this glycoprotein to THP-1 cells. Comparison of the binding capacity of B-LF and H-LF showed that B-LF more strongly binds to THP-1 cells, although both B-LF and H-LF bind to the same molecules in THP-1 cells. Further analysis to characterize the specific binding of B-LF to THP-1 cells is now in progress.

Poster Presentations - Field C1. Biochemistry

[PC1-1] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

SELECTIVE INHIBITION OF HUMAN CYTOCHROME P450 1A1 BY 3, 3',4, 5, 5'-PENTAMETHOXYSTILBENE

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Recently we have suggested that various hydroxystilbene compounds from natural sources showed strong inhibition of activities of human P450 1 isozymes such as CYP1A1, 1A2, and 1B1 (Chun, Y. J., Ryu, S. Y., Jeong, T. C., and Kim, M. Y. Drug Metab. Dispos. 29:1-6, 2001). Here we reported that 3,3',4,5,5'-pentamethoxystilbene (PMS), a synthetic stilbene compound, exhibited a potent and selective inhibition of human CYP1A1 with an IC50 value of 0.14μM. PBS showed 6700-fold greater selective inhibition of CYP1A1 over CYP1A2 (IC50=934μM) and 23-fold selectivity for CYP1A1 over CYP1B1 (IC50=3.2μM). PMS did not show any significant inhibition of ethoxyresorufin O-deethylation (EROD) activity in human liver microsomes. To elucidate the mechanism of inhibition by PMS, kinetic studies were performed. Analysis of the mode of inhibition indicated mixed-type inhibition of CYP1A1. The inhibition by PMS was not mechanism-based. The trapping agents glutathione, N-acetylcysteine, or dithiothreitol prevented the inhibition. Taken together, PMS is one of the most selective inhibitor of human CYP1A1 and may be considered as a good candidate for a cancer preventive agent in human.

[PC1-2] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Identification of a nucleolus protein, hNopp140, as a specific binder to doxorubicin by an affinity selection method with a phage display library

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Doxorubcin is a widely used anti-cancer drug that has cytotoxic activity against various types of cancer cells. DNA intercalation was assumed as one of the mechanism of the drug, however, the precise target and the mechanism of cytotoxicity of doxorubicin were not fully revealed. To examine the potential target protein against doxorubicin, we have used a biopanning method with a T7 phage library expressing human liver cDNA on the surface of phage. The phage library was screened against the immobilized doxorubicin, and a phage clone was isolated. Sequence analysis showed that the cloned phage displayed the C-terminal region of hNopp140 that had an important role in the biogenesis of nucleolus as well as cell division. When the cloned region of hNopp140 was expressed in E. coli and purified, it could be phosphorylated by casein kinase II and oligomerized in the presence of magnesium and fluoride ions as in vivo state. In addition, it interacted specifically to doxorubicin with apparent dissociation constant of 4.5 ´ 10-6M. Interestingly, doxorubincin bound to only the native form of purified protein not to the phosphorylated form. The significance of the interaction between doxorubicin and hNopp140 with relation to the cytotoxic activity of doxorubicin was discussed.

[PC1-3] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Proteomic study of ginsenoside-Rg1 (G-Rg1) in NIH3T3 mouse fibroblast cells.

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We have studied an activation mechanism of pp60c-src protein tyrosine kinase (PTK) by ginsenoside-Rg1 (G-Rg1) in NIH3T3 mouse fibroblast cells using proteomic technique. It was previously reported that G-Rg1 stimulated the activation of c-src kinase at 20 M with a 18hr-incubation, increasing the activity by 2-4-fold over that of untreated control with an increased cell proliferation. In the present study, we examined effects of G-Rg1 on pp60c-src protein tyrosine kinase(PTK) activity using a 2D