[PC2-11] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

The *ermK* leader peptide alterations leading to differential efficiency of induction by erythromycin

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The *ermK* gene from *Bacillus lichenformis* encodes an inducible rRNA methylase that confers resistance to the macrolide-lincosamide-streptograminB antibiotics. The *ermK* mRNA leader sequence has a total length of 357 nucleotides and encodes a 14-amino acid leader peptide together with its ribosome binding site. The secondary structure of *erm* leader RNA and a leader peptide have been reported as the elements that control expression. In this study, the contribution of specific leader pepetide amino acid residues to induction of *ermK* was studied using the PCR-based megaprimer mutation method. *ermK* methylases with altered leader peptide codons were translationally fused to *E.coli* β-galactosidase as reporter gene. The deletion of the codons for Thr-2 through Ser-4 reduced inducibility by erythromycin, whereas that for Thr-2 and His-3 was not. The replacement of the individual codons for Ser-4, Met-5 and Arg-6 with termination codon led to loss of inducibility, but stop mutation of codon Phe-9 restored inducibility by erythromycin. Collectively, these findings suggest that the codons for residue 4, 5 and 6 comprise the critical region for induction. The stop mutation at Leu-7 expressed constitutively *ermK* gene. Thus, ribosome stalling at codon 7 appear to be important for *ermK* induction.

[PC2-12] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Pathogenic and immunological properties of alcohol dehydrogenase in *Streptococcus pneumoniae*

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Despite the use of antibiotics and vaccines, *Streptococcus pneumoniae* remains a serious cause of morbidity and morality in human. In this study, pathogenic and immunological natures of alcohol dehydrogenase (ADH) in *S. pneumoniae* were elucidated. In vitro cytotoxicity test determined by lactate dedydrogenase release from A549 cells revealed that adh mutation significantly reduced cytotoxicity although in vivo intraperitoneal challenge of the adh mutant to BALB/C mouse exhibited marginal increase of survival time than the wild type. To determine the underlying mechanism of reduced virulence in the adh mutant, expression of virulence associated proteins (pneumolysin, choline binding protein, pneumococcal surface protein A, etc) was determined by Western blot analysis. Expression of virulence associated proteins in the adh mutant was not changed, however, autolysis upon exposure to penicillin and expression of autolysin (LytA) in the adh mutant were decreased than those of the wild type. Since all of the 29 clinical isolates of S. pneumoniae cross-reacted with antibody raised against ADH, these results suggest that ADH is highly conserved and would be one of the virulence factors in *S. pneumoniae*.

[PC2-13] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Isolation of α-Glucosidase Inhibitor Producing Soil Microorganism and Inhibitory Effects of Microbial Metabolites on α-Glucosidase

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To find α-Glucosidase Inhibitors produced by Actinomycetes, 20 soil samples were tested and 53 Actimycetes were isolated. One of 53 Actinomycetes (strain PM718) showed very potent inhibitory activity in vitro. The

morphological and physiological characteristics of strain PM 718 were investigated. The spore morphology, spore chain morphology and spore surface were observed by scanning electron microscope. The inhibitory activity of strain PM718 in vivo has been studied in mice made hyperglycemia by Streptozotocin treatment. The strain PM718 showed significant reduction of blood glucose level (more than 30%) in mice loaded with maltose.

[PC2-14] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Mn^{2+} dependent ClpL ATPase in *Streptococcus pneumoniae*

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HSP100/Clp family functions as molecular chaperone and ATP dependent protease. The *Streptococcus pneumoniae* ClpL, a homologue of bacterial ClpB and yeast cytosolic HSP104, is one of major heat shock proteins but its biochemical properties are unknown. In this study, ClpL in *Streptococcus pneumoniae* was characterized using histidine tagged recombinant ClpL. When ATP hydrolysis activity was compared in the presence or absence of a variety of nucleotides or divalent ions, either ATP or Mn^{2+} ion was found to increase significantly the rate of ATP hydrolysis. Furthermore, glutaraldehyde cross-linking and subsequent native-PAGE analysis showed that ClpL forms dimer, but in the presence of 4 mM concentration of Mn^{2+} ion, ClpL was aggregated. Thus ClpL seems to require Mn^{2+} ion as a cofactor for ATP hydrolysis and oligomerization *in vitro*.

Poster Presentations - Field C3. Cell Biology

[PC3-1] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Vitamin K Antagonist, NQ12 Inhibits PDGF-BB-Induced MAP Kinases Activation in Rat Aortic Vascular Smooth Muscle Cells

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Several 1,4-naphthoquinone derivatives have been reported to possess many pharmacological effects such as anti-viral, anti-fungal, anti-cancer and anti-platelet activities. We have reported that 2-chloro-3-[4-(ethylcarboxy)-phenyl]-amino-1,4-naphthoquinone(NQ12) had a potent inhibitory effect on the platelet aggregation in vitro and thrombosis in vivo. However, little has been known about functional roleof NQ12 on vascular smooth muscle cells (VSMCs).
In this study, we examined a possible antiproliferative effect of NQ12 on rat aortic vascular smooth muscle cells (VSMCs). NQ12 (1-5 μM) significantly inhibited the PDGF-BB-induced proliferation in a dose-dependent manner on rat aortic VSMCs. We also examined the intracellular signaling effect of NQ12 on the PDGF-BB-induced activation of mitogen-activated protein kinase (ERK1/2) by western blotting in cultured rat VSMCs. Pretreatment of rat VSMCs with NQ12 resulted in a significant inhibition of the PDGF-BB-induced ERK1/2. There was no evidence of cellular toxicity or apoptosis of NQ12 (5μM) as determined by trypan blue exclusion assay, flow cytometric analysis and DNA fragmentation assay.
These results suggest that the antiproliferative effects of NQ12 may be exerted by the inhibition of the PDGF-BB-induced ERK1/2, which can contribute to prevent atherosclerosis by inhibiting VSMCs proliferation.

[PC3-2] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Involvement of Akt in naphthoquinone analog-induced apoptosis in HL-60 cells