OL-030 Cloning of eae genes of Escherichia coli O157:T in pGEMT easy vector as DNA vaccine candidate
M. Golshan1,*, M. Kargar1, A. Doosti2. 1Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran, 2Biotechnology Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Background: Escherichia coli O157:H7 is associated with hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic-uremic syndrome in humans. In addition to producing Shiga-like (Vero) toxin and enterohemolysin, E. coli O157:H7 has been shown to attach to the cytoplasmic membranes of intestinal epithelial cells, to efface their microvilli, and to cause actin to accumulate beneath sites of bacterial attachment. These features are shared with several other enterohemorrhagic E. coli (EHEC) serotypes and members of the enteropathogenic E. coli (EPEC) group. The eae gene, which has been shown to be necessary for attaching and effacing activity, encodes a 94- to 97-kDa outer membrane protein (OMP) which is termed intimin. This gene is located in a chromosomal pathogenicity island also known as the locus of enterocyte effacement (LEE). The goal of this study was cloning of eae gene of E. coli O157:T in E. coli TOP10F strain.

Methods: The eae gene was isolated from total genome of E. coli O157:T by PCR and eae specific PCR primers. DNA fragment of eae gene was cloned by T/A cloning technique in pGEMT easy vector (Invitrogen, San Diego, Calif.), and this construct transformed into E. coli TOP10F strain.

Results: The results show that, eae was cloned in E. coli successfully. The sequencing result confirm that eae gene was cloned is correct and BLAST outcome demonstrated the sequences of eae is approved.

Conclusions: Therefore it seems that the DNA construct that was produced in this study can be used for DNA vaccine against eae of E. coli O157:T in future researchs.

OL-031 In vivo investigation of Killed Leishmania Vaccine’s (KLV) efficacy with Imiquimod (IMQ) as adjuvant in inhibition of visceralization of Leishmania major in Balb/c model
E. Salehizadeh1, H. Nahrevanian2,*, M. Farahmand2, R. Hajhosseini1, M.H. Alimohammadian3, R. Saghiri1, S. Naeimi1. 1Payame Nour University, Tehran Unit, Tehran, Iran, 2Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran, 3Department of Immunology, Pasteur Institute of Iran, Tehran, Iran, 4Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran

Background: KLV have been applied for its immunogenicity in human and mice model. IMQ, as adjuvant is inducing humoral and cellular immune responses during leishmaniasis. In this study, both KLV and IMQ were applied in order to investigate the inhibition rate of L. major replication and visceralization in mice.

Methods: Promastigotes of L. major were harvested from culture, counted and used to infect Balb/c mice. Primarily, mice were injected with KLV/IMQ, and then they were infected by L. major intradermally with 2×106 promastigotes. Six weeks after infection, a small nodule was appeared leading to a large lesion and visceralization. Effects of KLV/IMQ, physiopathological changes, lesion size, delay of lesion formation, proliferation of amastigotes inside MQs and detection of amastigotes in target organs were also studied.

Result: Data analysis of body weight, rate of hepato/splenomegaly, and survival rate indicated no significant differences among experimental groups. It is concluded that both KLV and IMQ represented no cytotoxic effects on the host, but they partly increased lesion size; and impressed number of amastigotes inside MQs. Application of KLV/IMQ decreased visceralization in liver and induced liver, spleen and plasma NO. Although, application of IMQ solely decreased visceralization in lymph nodes, but KLV/IMQ represented no effects in concentrations of plasma Cu/Zn and it increased liver SGOT and SGPT.

Conclusion: Unlike topical application of IMQ, injectable IMQ presented no ameliorative affects on CL. IMQ efficacy may be associated with route, dose and number of injection, which require more investigations.

OL-032 Expression of mRNA and protein of Fas associated death domain protein in fulminant hepatic failure model and the relationship of hepatocytes apoptosis
H.Y. Ge, Z. Zhen*. Department of infection disease, The Third Hospital of Hebei Medical University, Shijiazhuang, China

Objective: It has been confirmed that Fas associated death domain protein (FADD) participated in many death signal pathways induced by death receptor and has an connection role in apoptosis signal pathway. To study the expression of protein and mRNA of Fas associated death domain protein (FADD) in fulminant hepatic failure (FHF) induced by D-galactosamine (D-GaIN)/lipopolysaccharide (LPS) and the relationship of hepatocytes apoptosis.

Methods: The sensitized Wistar rat in the model group was injected by LPS and D-GaIN to induced fulminant hepatic failure; the control group was given the same volume physiological saline. The expression of protein and mRNA of FADD were detected by immunohistochemistry and RT-PCR and hepatocyte apoptosis was examined by common HE stain and flow cytometry.

Results: The liver histopathology showed apoptosis body and infiltration of inflammatory cells after 4 h of intraperitoneal injection of D-Gal/LPS, the hepatocyte apoptosis reached the peak at 12 h. At 24 h hepatocyte apoptosis evidently decreased subsequent hepatocytes necrosis occupied the important position. The apoptotic rate reached to 27% at 12 h. The expression of protein and mRNA of FADD prominent were dramatically increased positively correliative with hepatocyte apoptosis in D-GaIN/LPS group compared with control group.

Conclusions: The expression of FADD remarkably increased in FHF and be closely related with hepatocyte apoptosis. Scientifically regulation of the expression FADD may play an important role in prevention and treatment of FHF.

Free Paper Presentation 5: Tuberculosis
Saturday, July 16, 2011, 11:45-13:15
Meeting Room 311B

PL-005 Bioprospecting for potent anti-tuberculosis compounds from microbial natural product library
H. Guo1, L.X. Zhang1,*. 1IMCAS, China

Mycobacterium tuberculosis is second only to AIDS as one of the world’s most significant and devastating pathogens, which has infected over one-third of world’s population, and causes 2 million deaths annually. In the face of the catastrophic synergy between HIV and TB and the emergence of MDR and XDR-MTB strains, new drugs for the chemotherapy of tuberculosis are urgently needed. Microbial Natural Products or their derivatives have been an important

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source for most antibiotics on the market today, including the first-line and salvage TB regimens, including rifampin, streptomycin, amikacin and etc. A set of Microbial Natural Product Library (MNPL) containing secondary metabolites from a unique collection of actinomycetes and fungal strains from un- or under-explored ecological niches in China has been constructed. A pilot screen for potential anti-TB compounds was conducted with a selection of 5,000 extracts samples from this MNPL, utilizing a GFP labeled M. bovis BCG based HTS model (Z’-factor 0.8, CV < 15%, throughput 60,000 wells/day). 80 out of the 5,000 extracts showed >90% inhibition against logistically growing BCG were further evaluated on M. tuberculosis H37Rv strains at Broad Institute, as a dilution series ranging from 1× to 1/128×. The results showed that 46 extracts demonstrated anti-H37Rv activity, with 8 showing activity at 1/16×, and 1 showing activity at 1/128×. The following large scale fermentation and bioactivity guided compound isolation work lead to the discovery of diversified class of anti-TB compounds, including actinomycins (MIC 1–4 µg/ml), quinomycins (MIC 0.5 µg/ml), nanaomycins (MIC 8 µg/ml), cyclopeptides (MIC 2–8 µg/ml), arthranaquiones (MIC 4–8 µg/ml), oligomycins, part of which were new compounds (not listed). 

**[DL-033] Etambutol-mediated changes in rat liver cytochrome P-450 isoforms expression and DNA-fragmentation processes**

S. Anisimova1*, G. Shayakhmetova1, L. Bondarenko1, V. Kovalenko1. 1SI "Institute of Pharmacology & Toxicology" National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine

**Objectives:** Etambutol is one of first-line antitubercular drugs in current therapeutic regimen. Its main disadvantage is wide spectrum of adverse effects which can lead to therapeutic failure. The aim of present study was to investigate etambutol effects on rat liver cytochrome P-450 isoforms expression and DNA-fragmentation processes.

**Methods:** Wistar albino male rats (160–200 g b.w.) were divided into two groups: I – received etambutol per os at a dose of 155 mg/kg b.w./day, II – control. After 60 days of the experiment, rats were sacrificed via cervical dislocation. Expression of rat liver CYP2E1, CYP3A2 and CYP2C23 were studied by RT-PCR methods. DNA fragmentation was investigated electrophoretically.

**Results:** Our data demonstrated etambutol-mediated quantitative and qualitative changes in male rat DNA fragmentation and expression of CYP2E1, CYP3A2 and CYP2C23 in comparison with control. CYP2E1 and CYP3A2 expression increasing was accompanied with CYP2C23 expression inhibition. DNA fragmentation processes were also greatly intensified at etambutol treatment. Its introduction caused appearance of new fractions with longer DNA-fragments (above 1000 b.p., 1000–800 b.p. and 800–600 b.p.). Among shorter DNA-fragments main fraction contained chains with 20–30 b.p.

**Conclusion:** Thus etambutol treatment caused adverse effects in organisms on the level of cytochrome P-450 isoforms expression and DNA-fragmentation processes. Extensive investigation of etambutol and cytochrome P-450 system interactions allowed to prevent or correct this antitubercular agent adverse effects.

**[DL-034] A comparison study of extrapulmonary and pulmonary tuberculosis in Hong Kong**

S.S. Lamb1*, I.F. Hung1, K.K. To1. 1Queen Mary Hospital, Hong Kong SAR, China

**Background:** This study examines the differences between culture positive extrapulmonary and pulmonary tuberculosis (TB) patients. It correlates these findings with the current understanding of extrapulmonary TB. It looks at how these factors affect mortality at six months and at the role of underlying diseases in drug resistance.

**Method:** This is a two year retrospective study comparing 115 extrapulmonary TB patients with 115 pulmonary TB patients.

**Results:** Extrapulmonary patients were younger than pulmonary patients with a median age of 53 years versus 73 years (p<0.001). They presented more commonly with lymphadenopathy (p<0.001). More extrapulmonary patients had chronic renal failure (p<0.002) and Human Immunodeficiency Virus (HIV) infection (p<0.005). They had a higher median erythrocyte sedimentation rate (61 mm/h vs. 48 mm/h, p=0.017), and lactate dehydrogenase level (309 U/L vs. 197 U/L, p=0.006). They had a lower median hemoglobin (10.7 g/dL vs. 11.7 g/dL, p=0.002), white blood cell count (7.4×10^9/L vs. 8.5×10^9/L, p=0.039), and lymphocyte count (0.9×10^9/L vs. 1.1×10^9/L, p=0.051).

There was no difference in mortality between extrapulmonary and pulmonary groups. Multivariate analysis identified age >60 years (Odds Ratio [OR] 2.4, 95% Confidence interval [95% CI] 0.98–5.77, p=0.054), presentation with decreased general condition (OR 3.5, 95% CI 1.46–8.34, p=0.005), hypertension (OR 2.7, 95% CI 1.15–5.38, p=0.021), radiological old TB (OR 2.6, 95% CI 1.25–5.52, p=0.014), and pleural effusion (OR 3.2, 95% CI 1.45–7.03, p=0.004) as independent risk factors for mortality.

9.1% of all cases had culture evidence of drug resistance. HIV infection (p=0.001) and intravenous drug usage (p=0.001) were the two risk factors identified.

**Conclusion:** There are important differences in demographics, clinical presentation and risk factors for extrapulmonary compared with pulmonary TB. Mortality is related to age and co-morbidity.