Secretion of hepatitis C virus E2 protein could enhance neutralization antibodies induced by DNA vaccine

Zhi-hui Chen1, Yi-min Tong2, Zhong-tian Qi*,2. 1Department of Infectious Diseases, Shanghai Hospital, Second Military Medical University, Shanghai, China; 2Department of Microbiology, Second Military Medical University, Shanghai, China

Objective: To explore the feasibility of induction of neutralization antibodies against HCV infection by DNA vaccines.

Methods: Two expression plasmids of HCV envelope 2 protein were constructed, plasmid pCI-1b746 encoding full length of E2 protein, and plasmid pCI-1b661 encoding hydrophobic carboxyl terminal truncated E2. 293T cells were transfected with either of both plasmids, respectively, and intracellular and secreted E2 protein were analyzed by Western blot or immunofluorescence. Then the plasmids were used to inoculate BALB/c mouse intramuscularly. The sera antibodies against E2 and hypervariable region 1 (HVR1) were detected by ELISA and the neutralization activity of the sera were assayed with HCV pseudotype particle (HCVpp).

Results: Both plasmids could express HCV E2 protein, the expression product of pCI-1b746 could not secrete, while that of pCI-1b661 could secrete into culture medium. The secretion of HCV E2 protein enhanced antibody response elicited by DNA vaccines in mice significantly. Sera from pCI-1b661 immunized mice showed stronger neutralization activity than that from pCI-1b746 immunized mice. For sera from pCI-1b661 immunized mice, the neutralization activity against HCVpp were positive correlation with anti-HVR1 antibodies levels.

Conclusions: Secretion of E2 protein by DNA vaccine can enhance the production of neutralization antibodies against HCVpp infection and the neutralizing activity is depended on the presence of HVR1 antibodies.

OL-004  
Suppression of liver fibrosis is associated with the decrease of transforming growth factor-β1 and increase of matrix metalloproteinase-1 expression after interferon-α therapy in chronic hepatitis C patients

Yun-ru Li*. Beijing Ditan Hospital, Beijing, China

Aim: To evaluated relationship of the changes of transforming growth factor-β1 (TGF-β1), matrix metalloproteinase-1 (MMP-1) and tissue inhibitors of matrix metalloproteinase-1 (TIMP-1) expression with liver fibrosis in liver tissue after IFN-α therapy in patients with chronic hepatitis C.

Methods: Eleven patients with chronic hepatitis C treated by IFN-α were divided into two groups on the outcome of therapy, including a complete responder group (CR) and a non-responder group (NR). Liver biopsy specimens were stained with hematoxylin and eosin (HE) and Masson’s trichrome staining. Liver fibrosis was semiquantitated by Modified Ishak scoring system.

Results: There was a significant reduction in Ishak fibrosis score in SVR group (P<0.05). The TGF-β1 expression was reduced and the MMP-1 expression was elevated in SVR groups after IFN-α therapy (P<0.05). The ratio of the density of MMP-1 to TIMP-1 expression increased in SVR group after IFN-α therapy compared with NR group (P<0.05). The reduction of fibrosis score correlated significantly with that of TGF-β1 expression in all patients (R=0.744, P<0.01). The increase of MMP-1 expression after IFN-α therapy significantly correlated with the decrease of fibrosis (R=0.925, P<0.05) and HAI (R=0.837, P<0.05) scores in SVR group.

Conclusion: IFN-α improves liver fibrosis in hepatitis C patients by decreasing TGF-β1 expression, as well as by increasing MMP-1 expression to degrade fibrosis.

OL-005  
Immunohistochemical study of hepatic oval cells in fetal liver and chronic C hepatitis

Maria Comanescu*,1, Violeta Comanescu2. 1University Clinical Hospital of Bucharest, Romania; 2County Clinical Hospital of Craiova, Romania

Background: Hepatic oval cells differentiate into two types of cells, hepatocytes and biliary cells. The aim of this study was to identify oval cells in fetal liver and in adult liver from patients with chronic C hepatitis.

Method: This study was performed using two study group. The first included 10 human fetal livers from embryos by therapeutic abortions with gestational ages ranging between 10 and 21 weeks. The second group included 30 liver biopsies from patients with viral C hepatitis. The liver specimens were fixed in formaldehyde, embedded in paraffin and cut at 5 μm. Slides were stained using standard (HE, VG) and immunohistochemical stainings (CK19, CD45, alfaSMA and desmin).

Results: All cases were examined by light microscopy. Oval cells were identified as small cells with basophilic cytoplasm and small oval nuclei, located in periportal areas, and in association with fibrosis and inflammation. They were positive for CK19 and negative for CD45. Desmin was positive in fetal hepatocytes and negative in adult hepatocytes. Alfa SMA was positive in perportal cells and negative in hepatocytes and biliary ducts. In chronic C hepatitis alfa SMA was positive in isolated cells in the areas of necrosis.
**OL-006**  Efficacy, tolerability and safety of personalized low-dose IFN treatment in patients with HCV-related liver cirrhosis and severe complications

Xiaoling Fan*, Wenyan Zhang, Yun-rui Li, Xiaojie Wang. Beijing Ditan Hospital, Beijing, PR China

**Object:** To observe the efficacy, tolerability and safety of personalized low-dose IFN treatment in patients with HCV-related liver cirrhosis and severe complications.

**Method:** Personalized low-dose IFN treatment was performed in 61 patients. Less than 3 million units of personalized low-dose natural IFN-alpha was administered QOD intramuscularly or less than 50 μg Peg-IFN alpha-2b QW intrasubcutaneously, which depended on patients tolerability, and plus Ribavirin 600 mg/day. The course of treatment was at least 24 weeks. Some patients received more than 2 years IFN maintenance therapy.

**Results:** Twenty of the 62 patients showed a rapid virological response in 4 weeks treatment (32.5%). Twenty eight of 62 patients showed a complete early virological response in 12 weeks treatment (45.16%). Thirty four of 62 patients showed HCV RNA undetectable in 24 weeks IFN therapy (54.83%). ALT levels normalized (about 40 IU/L) at the end of 24 weeks therapy. 11 of 25 patients had a sustained virological response who were given more than 2 years Maintenance Therapy (44%). Definitive discontinuation of therapy was necessary in 7 patients (11.29 %) because of side effects.

**Conclusion:** Personalized low-dose IFN and ribavirin combination therapy was useful and safe in patients with HCV-related liver cirrhosis and severe complications for whom standard-dose interferon and ribavirin combination therapy was difficult.

**Free Paper Presentation 2 – Bacterial Infections/Antibiotics I**

**OL-007**  Carbapenems resistance in Gram-negative bacilli isolates in an intensive care unit

Eleni Antoniadou1, Spyridoula Vasilikou1, Nikolaos Voloudakis2, Savvato Tsingene3, Asimoula Kotei3. 1Intensive Care Unit, 2G. Gennimatas General Hospital of Thessaloniki; 3Medical School, University of Crete, Heraklion; 4Microbiology Laboratory, “G. Gennimatas” General Hospital of Thessaloniki

**Objective:** To determine resistance of Ps. aeruginosa, A. baumannii and K. pneumoniae as prevalent nosocomial agents to commonly used antibiotics including imipenem, meropenem and ertapenem.

**Methods:** Identification of microorganisms and susceptibility test was performed with the Vitek 2 (BioMerieux®, France) and the carbapenems resistance and EDTA-containing discs was employed. Quality control was ensured by keeping weekly records of disk diffusion Ps. aeruginosa (ATCC 27853). MIC values for carbapenem were determined by the E-test (AB Biodisk, Solona, Sweden) as recommended by manufacturer. K. pneumoniae ATCC 70603 was used as a positive ESBLs strain.

**Results:** Information was available on antibiotic susceptibility of 1044 gram-negative bacteria, of which the most common were A. baumannii 414, Ps. aeruginosa 328, K. pneumoniae 169. No duplicate isolates from the same patients were included. All microorganisms were isolated from tracheal tube aspirates, urine, wound, blood and other sterile body fluids. The resistance rates (%) of A. baumannii, Ps. aeruginosa, and K. pneumoniae were: imipenem 76/61/67, meropenem 68/52/65, ertapenem 77/65/68, amikacin 88/48/40, piperacillin/tazobactam 82/35/67, ceftazidime 100/68/65, ciprifloxacin 92/64/15, aztreonam 100/84/72. Of 169 isolates of K. pneumoniae 92 (54.43%) were ESBLs.

**Conclusions:** The most isolates of A. baumannii were multi-drug resistant. The majority of isolates were resistant to 5 or more antibiotics tested and some strains were defined by resistance to all antimicrobial agents except colistin. The resistance to carbapenems rose dramatically.

**OL-008**  Molecular characterization of extended-spectrum β-lactamases and AmpC enzymes in Enterobacteriaceae in Beijing, China

Lingxian Zhu1,2, Di Jiang1,2, Zhiwei Zhang1,2, Can Wang1,2, Jing Cheng1,2. 1National Engineering Research Center for Beijing Biochip Technology; 2CapitalBio Corporation, PR China

**Objectives:** A study was conducted to evaluate the molecular characterization of extended-spectrum β-lactamases (ESBLs) and plasmid-mediated AmpC enzymes in Enterobacteriaceae in Beijing, China.

**Methods:** Production of ESBLs and plasmid-mediated AmpC among 240 non-duplicate Enterobacteriaceae isolates was screened by the phenotypic methods and the molecular methods. The epidemiological relationship of the isolates was studied by random amplified polymorphic DNA (RAPD) analysis.

**Results:** CTX-M type ESBLs were the most prevalent ESBLs. Three E. coli isolates simultaneously harbored blaCTX-M-3 and blaCTX-M-9 genes. SHV-12 was the most prevalent SHV-type ESBL. SHV-2a, SHV-2 and SHV-5 ESBLs, and SHV-27 and SHV-44 non-ESBLs were detected. Two Klebsiella pneumoniae isolates expressed a novel ESBL, SHV-43a, which had one substitution (Leu35Gln) compared with SHV-43. DHA-1 was the most prevalent plasmid-mediated AmpC enzyme, found mainly in K. pneumoniae (n=11). We also identified the plasmid-mediated CMY-2 enzyme in two E. coli isolates. RAPD analysis revealed that 53 CTX-M-13- and 7 CTX-M-3-producing E. cloacae isolates recovered from a single hospital exhibited a high similarity of RAPD patterns, indicating clone-related spread.

**Conclusions:** This survey indicates the high frequencies of CTX-M-9/3 ESBLs and plasmid-mediated DHA-1 in China, reports the first emergence of DHA-1-producing E. cloacae and interhospital epidemic CTX-M-13/3-producing E. cloacae.

**OL-009**  Virulence factors determination and molecular characterisation of Malaysian Vibrio cholerae

Cindy Shuan Ju Teh*, Kwai Lin Thong. Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia

**Objective:** To determine the virulence profiles of Malaysian Vibrio cholerae and to investigate the relatedness of the strains using molecular typing methods.

**Methods:** 43 V. cholerae were isolated from clinical and environmental sources. Strains isolated were serogrouped and PCR were carried out for determination of the virulence genes harbored in each strain. The strains were further characterized using molecular subtyping methods such as RAPD, ERIC, REP-PCR and PFGE fingerprinting to investigate the relatedness among the strains.

**Results:** Twenty-three O1, one O139 and 19 non-O1/nonO139 strains were isolated. All but one O1 strains harbored virulence genes such as ctxA, zot, rtXa, rSrR, toxT, toxR, tcpA, tcpC, and