Establishment of highly efficient full-length HCV 1b genome cell culture system by inserting long distant structural fragment into replicon

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Objectives: To establish a full-length genome of hepatitis C virus (HCV) 1b, the dominant strain in China, cell culture system for further study.

Methods: The 5'-end of half HCV-1b genome (5.2kb) was amplified from Chinese chronic hepatitis C patients' sera samples with the refined long distant RT-PCR technique. The full length recombinant plasmid of HCV 1b was constructed by inserting the long distant structural regions directly into HCV 1b replicon containing the non-structural regions. In vitro transcribed genomic HCV RNA was transfected into Huh7.5.1 cells by liposome-mediated method. The real time quantitative RT-PCR, Western Blot, inoculation of naive Huh7.5.1 cells, immune fluorescence and titration of infectious HCV were used for identification of HCV replicon and presence of infectious virion.

Results: The real time quantitative RT-PCR assay revealed the highest titer of HCV was 6.5 × 10^7 copies/mL in the cultural supernatants. While both Western Blot analysis and immune fluorescence confirmed the expression of HCV core protein in the transfected cells. Subsequent infection of naive Huh7.5.1 cells with supernatant of HCV cell culture resulted in high levels of HCV proteins and RNAs.

Conclusions: These results demonstrate the successful establishment of a HCV 1b culture system by the new strategy of inserting long distant structural amplicon into replicon that produces infectious virus, which will allow the study of each aspect of the entire HCV life cycle and related studies.

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**PP-147** Construction of a chimeric GB virus B with hepatitis C virus NS2-NS4A

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Background: HCV infection became a worldwide threat to human health, which can lead to chronic hepatitis C, cirrhosis and hepatoma. As only human and chimpanzees are susceptible to hepatitis C virus, the progress of HCV research has been obstructed due to the absence of a reliable small primate animal model. GB virus B (GBV-B), being very close to hepatitis C virus (HCV) phylogenetically, can replicate effectively in vivo of common marmosets, that has been made an attractive surrogate virus for HCV replication study. It was reported that HCV NS2-NS3 protease, NS3 protease and NS4A complex are critical for virus maturation and replication. The construction of chimeric GB Virus B with Hepatitis C NS2-NS4A region can be used to develop a marmoset model for antiviral and immune studies.

Methods: RT-PCR and overlapping PCR were applied to complete jointing gene fragments and T7 transcription kit was used to produce chimeric GBV-B/HCV infectious RNA in vitro.

Results: A chimeric clone originating from GB virus B (GBV-B), in which GBV-B NS2-NS4A region are replaced by analogous sequence of the HCV genome, was constructed.

Conclusions: This chimeric clone lays the roots for a surrogate model of Hepatitis C virus, insights into HCV replication mechanism and further HCV NS3 epitope-based research.

**PP-148** Comparisons of molecular responses between recovery and chronic HCV infection from blood donors in Beijing and Guangdong, China

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Background: Hepatitis C virus (HCV) infection is a major public health problem in China. However systematic studies conducted on mechanism of recovery and chronic infection with HCV are little known. A detailed study was carried out to investigate IFN-α and IFN-γ correlating to antibody reactivity and viral factors in the population of recovered and chronic HCV infection from blood donors.

Methods: 160 plasmas samples reactive with anti-HCV assays were collected from blood donors in Guangdong and Beijing. HCV antibody reactivity was presented as S/CO by at least three EIA assays. All samples were tested for ALT and viral load, confirmed by nested-PCR, and classified as three statuses of recovery (RNA-/Ab+), chronic (RNA-/Ab+) and false positive (RNA-/Ab+) infections. Productions of IFN-α and IFN-γ in the serum were also quantified by ELISA. The genotypes of HCV from chronic samples were phylogenetically analyzed with 5′-NCR sequences (215–218bp).

Results: 45 recovery, 76 chronic and 39 false positive of 160 HCV antibody positive plasmas were finally confirmed. The rate of recovered HCV infected individuals in blood donors was 37.2% proximately. 63 HCV strains were genotyped,
including 42.9% genotype 1, 14.3% genotype 2, 19.0% genotype 3 and 23.8% genotype 6. Genotype 1 was predominant in Beijing, while genotype 6 was mainly detected in Guangdong. The levels of IFN-γ production and antibody reactivity from chronic infection donors were significantly higher than those from recovery and false positive infection donors (p < 0.001), but no statistical difference of IFN-α among populations from three HCV infection statuses was observed.

**Conclusion:** IFN-γ but IFN-α may play a role paralleled with antibody in viral clearance during natural history of HCV infection.

**PP-149 Seroprevalence of anti-HCV in children living in Ulaanbaatar, Mongolia**

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**Background:** Our previous study established that HCV infection is wide-spread in Mongolia. So, many Government and non-Government measures on HCV prevention are non-specific. So, there is needed to check the results of the measures.

**Objective:** To assess prevalence of HCV infection in Mongolian children.

**Methods:** We randomly selected 199 "healthy" children (ages 1–15) in May 2009, from Ulaanbaatar, matched by age and sex and tested serum of all subjects for anti-HCV.

**Results:** Eighteen of 193 serum samples tested positive for anti-HCV (9.3%). Older age was more likely to show a positive test (presented in the table by age and sex).

<table>
<thead>
<tr>
<th>Age</th>
<th>Subjects</th>
<th>Anti-HCV</th>
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<tbody>
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<td>Total</td>
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**Discussion and Conclusion:** More half the population of Mongolia lives in Ulaanbaatar. We have carried two studies on the prevalence of HCV infection in children in Ulaanbaatar (1998, 2002). These studies did not showed a significant difference in HCV seroprevalence between rural and urban subjects. As the results, we estimate the prevalence of HCV infection in Ulaanbaatar to the figure for the whole Mongolian population. The seroprevalence, found in this study is significantly higher compared with our previous study. Therefore, all measures to help prevent HCV infection in Mongolia should be intensified.

**PP-150 Evaluation of serum TIMP-1 & TGF-β1 in Egyptian patients with chronic hepatitis C, genotype 4 & chronic hepatitis B infection**

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**Background:** HCV & HBV infections are known to be major public health problem in Egypt. As chronic liver disease progresses, an imbalance occurs between synthesis and breakdown of extra cellular matrix (ECM). Matrix metalloproteinases (MMPs) are involved in degrading ECM while tissue inhibitors of metalloproteinases (TIMPs) prevent their fibrolytic action. Transforming growth factor β1 (TGF-β1) is a key mediator of liver fibrogenesis. Aim of this study is evaluating TIMPs & TGF-β1 as fibrogenic seromarkers in comparison to histopathological examination of liver.

**Methods:** Commercially available ELISA assays were used to study circulating levels of TIMP-1 & TGF-β1 in 50 patients with chronic hepatitis C patients and 20 patients with chronic hepatitis B compared to 10 healthy control subjects.

**Results:** In HCV patients, TIMP-1 was elevated with more progressive liver disease with a sensitivity of 100% the specificity of 87%. Serum level of TGF-β1 in chronic hepatitis (B) was slightly decreased from healthy control without significant correlation. Its mean and standard deviation was (157.81±44.1) and (164.18±42.22) in the HBV and control group respectively.

**Discussion and Conclusion:** TIMP-1 is a reliable predictor to detect advanced fibrosis, in HCV patients genotype 4.

**PP-151 Increased PD-L1 expression and PD-L1/CD86 ratio on dendritic cells were inversely associated with impaired dendritic cells function in HCV chronic infection**

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**Background:** Hepatitis C virus (HCV)-specific T cell immunity was impaired in HCV chronic infection and resulted in the persistency of HCV infection. Dysfunction of dendritic cells (DCs) was believed to be involved in this T cell exhaustion, but the mechanisms were rarely understood.

In this study, we evaluated surface costimulatory marker (CD83, CD86, and CD40) and coinhibitory marker (PD-L1) expression and allostimulatory capacity of plasmacytoid DCs and myeloid DCs in twenty five HCV-infected patients compared to twenty healthy controls. All patients and control were seronegative for anti-HIV and anti-HBV.

DCs were isolated from PBMC by use of BDCA-1 positive cell isolation kit for myeloid DCs and BDCA-4 positive cell isolation kit for plasmacytoid DCs according to the manufacturer’s instructions.

We found both costimulatory marker and coinhibitory marker expression were decreased in HCV-infected patients compared with healthy control. The ratio of PD-L1 versus CD86 was also increased in HCV-infected patients and the ratio positive correlated with PD-L1 expression on DCs. Allostimulatory capacity of DCs was impaired in HCV infection and this allogeneic MLR was inversely correlated with PD-L1 expression and PD-L1/CD86 ratio.

**Discussion and Conclusion:** These findings suggested that the effect of inhibitory marker PD-L1 overwhelmed the effect of costimulatory markers and negatively regulated DC-T activation. Our finding will be helpful to understand the mechanism of dysfunction.