Human HepaRG cells Support Long Term Propagation of Hepatitis C Virus (HCV): Candidate Infection System for Screening Entry Inhibitors

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BACKGROUND

Anti-HCV E1/E2/D32.10: A new neutralizing monoclonal antibody

Relevant unique properties of the mAb D32.10:

- Immunization of mice with HCV particles derived from the serum of chronically infected patient (HCVosp = immunized)
- Specific recognition of E1/E2 envelope complex expressed on the surface of natural HCVosp
- High conservation of the 3 region E1, E2A, E2B recognized by D32.10 (genotype 1a, 1b, 2a, 3a, 3b)
- E2A and E2B encompass C2FT binding site (mutagenesis in 2008)
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- E2B is CD4+ T cell site (Park et al., 2007)
- E2B is CD8+ T cell site (Park et al., 2007)

HepaRG hepatocytes: A new human progenitor cell line

Unique characteristics:

- A bipotent progenitor cell line
- A metabolically competent human cell line, suitable for high throughput screening
- A good in vitro model for developing biotransformation and metabolic assays
- Exhibits hepatocyte-like morphology
- Exhibits a large set of liver-specific functions (close to primary hepatocytes)
- Exhibits stable drug metabolizing enzyme activities along sub-cultures
- Stable and subnuclear cytochrome

AIMS of the study

- Investigate whether progenitors and/or differentiated HepaRG cells could be directly infected with HCVosp and sustainably propagate HCV RNA-containing enveloped particles
- Further assess the anti-E1 E2 D32.10 mAb neutralizing properties in vitro

METHODS AND RESULTS

1. Inhibition of the Binding (IB) of HCVosp to Human Hepatocytes by the anti-E1E2 mAb D32.10

   Methods (1):
   - In vitro direct binding assay
   - Radiodetection (RIA) of HCVosp

   Results (1):
   - Inhibitory effect mediated by genotype 3
   - Inhibitory effect mediated by genotype 3

   Conclusions (1):
   - Anti-E1E2/D32.10 mAb efficiently neutralizes HCVosp
   - Anti-E1E2/D32.10 mAb efficiently neutralizes HCVosp

2. Infection of HepaRG cells with HCVosp (genotype 3): Inhibition by the anti-E1E2 mAb D32.10

   Methods (2):
   - Infection of HepaRG cells with HCVosp
   - Detection of HCVosp particles

   Results (2):
   - Inhibitory effect mediated by genotype 3
   - Inhibitory effect mediated by genotype 3

   Conclusions (2):
   - Inhibitory effect mediated by genotype 3
   - Inhibitory effect mediated by genotype 3

CONCLUSIONS & PERSPECTIVES

- HepaRG progenitor cells are permissive to HCV infection
- Differentiated HepaRG cells support long term propagation of infectious lipoprotein-associated enveloped authentic patient-derived HCV particles
- Anti-E1E2/D32.10 mAb efficiently neutralizes (50%) the infection only in the HCVosp-HepaRG system

The HCVosp-HepaRG cellular model reflects the in vivo situation and could be adapted as a standardized infection system using cryopreserved HepaRG® from Biopredic (differentiated HRF16 or culture KIT-0901) for the screening of entry inhibitors.