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

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BACKGROUND

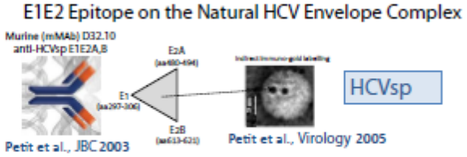
Anti-HCV E1E2/D32.10 : A new neutralizing monoclonal antibody

Relevant unique properties of the mAb D32.10 :

- Immunization of mice with HCV particles derived from the serum of chronically-infected patient (HCVsp = immunogen)
- Specific recognition of E1E2 envelope complexes expressed on the surface of natural HCVsp
- High conservation of the 3 regions E1, E2A, E2B recognized by D32.10 (genotype 1a, 1b, 2a, 3a)
- E2A and E2B encompass CD81-binding sites (Rothwangl et al. 2008)
- E2A and E2B encompass GAG-binding sites (Olenina et al., 2005)
- E1 is CD4 T cell site (Von Hahn et al., 2007)
- E2B is CTL epitope (Sarobe et al., 2001)

Transfer Technology Office : INSERM-Transfert, Paris, France
Patent PCT-EP 2004/003412; EP n° 200480008734.9; US n° 10550295; Divisional application n° 12/408 080 (20/03/2009)

E1E2 Epitope on the Natural HCV Envelope Complex



Discontinuous antigenic determinant on enveloped HCV serumderived particles (HCVsp)

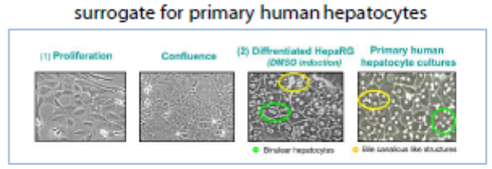
HepaRG hepatocytes : A new human progenitor cell line

Unique characteristics :

- A bipotent progenitor cell line Parent, Petit et al. Gastroenterology2004
- A metabolically competent human cell line, suitable fro high throughput screening
- A good in vitro liver model for developing biotransformation and metabolic assays
- Exhibit hepatocyte-like morphology
- Exhibit a large set of liver-specific functions (close to primary hepatocytes)
- Exhibit stable of drug-metabolizing enzyme activities along sub-cultures
- Stable and subnormal caryotype

(Gripon et al. 2002; Cerec et al. 2007; Lübberstedt et al. 2010)

surrogate for primary human hepatocytes



Patent WO 03004627: P. Gripon (PS), S. Rumin (PA), C. Guguen-Guillouzo (PS), C. Trépo

attractive candidates for studying HCV-host interactions

AIMS of the study

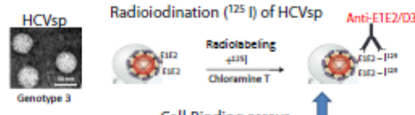
- ☒ investigate whether progenitors and/or differentiated HepaRG cells could be directly infected with HCVsp and sustainably propagate HCV RNA-containing enveloped particles
- ☒ further assess the anti-E1E2 D32.10 mAb neutralizing properties in vitro

METHODS AND RESULTS

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

Methods (1)

In vitro direct cell-binding assay:



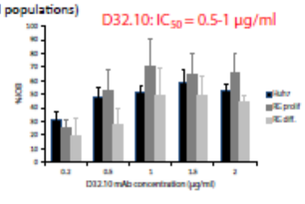
Cell-Binding assays

Scatchard Plot Analysis (site number; affinity) ← HUMAN HEPATOCYTES: Huh-7 hepatoma cells, HepaRG at proliferating or differentiated stages

Inhibition of binding (IOB) experiments : + anti-E1E2/D32.10 mAb (increasing conc.: 0.2 to 200 µg/ml)

Results (1)

HCVsp (all populations) D32.10: IC_{50} = 0.5-1 µg/ml



Inhibitory effect independent of genotype (1a or 3)

Conclusions (1)

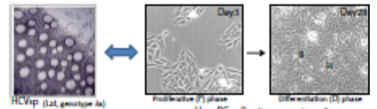
- ☒ Conformational E1E2/D32.10 epitope involved specifically in HA-interactions (low Kd) between HCVsp and hepatocytes
- ☒ mAb D32.10 = Efficient highly specific IOB effect

Cf. Ndongo , Drouet, Petit et al. J. Med. Virol. 2009

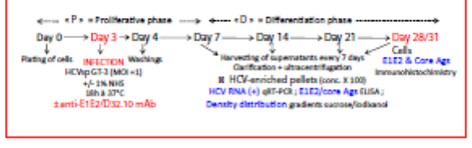
(2) Infection of HepaRG cells with HCVsp (genotype 3) : Inhibition by the anti-E1E2 mAb D32.10

Methods (2)

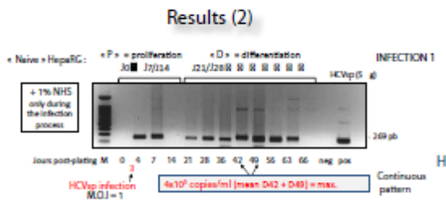
Infection system of the human HepaRG cell line with HCVsp:



Primo-infection experimental protocol (INFECTION 1 & 3) :

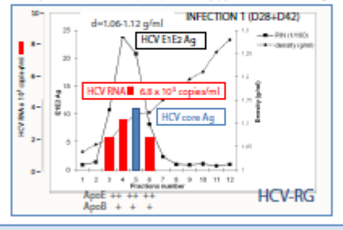


Results (2)

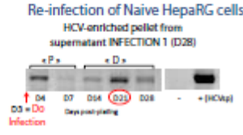


Production of E1E2/core/RNA(+) infectious HCV particles

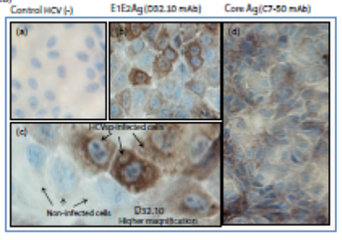
The density distribution of E1E2/core/HCV RNA(+) particles in Iodixanol gradient



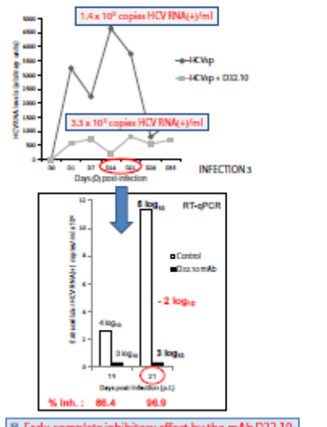
Re-infection of Naive HepaRG cells



HCV E1E2 and core protein detection in HCVsp-infected HepaRG cells (Immunohistochemistry)



Inhibition of the D32.10 mAb on virion RNA production in HepaRG culture supernatants



Methods (2) — Primo-infection experimental protocol (INFECTION 1 & 3) :

- ☒ HepaRG cells in a proliferative phase (Day 3 post plating) = dedifferentiated, depolarized epithelial phenotype
- HCV infection settling
- HepaRG cells in a differentiated phase (Day 21 to Day 66 post plating) = mature hepatocyte phenotype(polarization, active Golgi-ER transport, increased flux of secreted proteins,..) Cf. Parent & Beretta, GenomeBiol. 2008
- HCV replication and propagation

Cf. Ndongo-Thiam , Trépo, Petit et al. Hepatology2011

Conclusions 2

- ☒ Correlation between the detection of HCV RNA (+), coreAg, E1E2Ag and ApoE/B
- ☒ Secreted HCV particles are infectious and sedimented at density = 1.06-1.12 g/ml in iodixanol gradient (corresponding to 1.17-1.21 g/ml in sucrose gradient)
- ☒ E1E2 and Core antigens accumulate in the cytoplasm with intense staining pattern
- 50-60 % of infected cells at day 28 p.i. (1-month p.i.)

- ☒ Early complete inhibitory effect by the mAb D32.10 (86 to 97%) in the HCVsp-HepaRG system
- ☒ INHIBITION OF HCVsp ENTRY

This suggests/supports neutralizing capability of the anti-E1E2-D32.10 mAb in vitro

CONCLUSIONS & PERSPECTIVES - Relevant messages

- ☒ HepaRG progenitor cells are permissive to HCV infection
- ☒ Differentiated HepaRG cells support long-term production of infectious lipoprotein-associated enveloped authentic patient-derived HCV particles
- ☒ Anti-E1E2 D32.10 mAb efficiently (0.5 µM) neutralizes (90%) the infection only in the HCVsp-HepaRG system

The HCVsp-HepaRG cellular model reflects the in vivo situation and could be adapted as a standardized infection system using cryopreserved HepaRG® from Biopredic (differentiated HRP116 or culture KIT901) for the screening of entry inhibitors.