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HEPATOLOGY

Opening the door for hepatitis C virus infection in genetically humanized mice

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10 **Opening the door for hepatitis C virus infection in genetically humanized mice**
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14 Thomas.Baumert@unistra.fr.
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19 **Abbreviations:** CLDN1: claudin-1; Fah: fumarylacetoacetate hydrolase; GFP: green
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21 fluorescent protein; HCV: hepatitis C virus; HCVcc: cell culture-derived HCV; HCVpp: HCV
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23 pseudoparticles; JFH1: Japanese fulminant hepatitis 1; OCLN: occludin; SCID: severe
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25 combined immunodeficiency; SR-BI: scavenger receptor class B type I; uPA: urokinase
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27 plasminogen activator
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3 Comment on:

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5 Dorner M, Horwitz JA, Robbins JB, Barry WT, Feng Q, Mu K, Jones CT, Schoggins JW,
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7 Catanese MT, Burton DR, Law M, Rice CM, Ploss A. A genetically humanized mouse model
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9 for hepatitis C virus infection. *Nature*. 2011 Jun 8;474(7350):208-11
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16 **Abstract**

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18 **Hepatitis C virus (HCV) remains a major medical problem. Antiviral treatment is only**
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20 **partially effective and a vaccine does not exist. Development of more effective**
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22 **therapies has been hampered by the lack of a suitable small animal model. Although**
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24 **xenotransplantation of immunodeficient mice with human hepatocytes has shown**
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26 **promise, these models are subject to important challenges. Building on the previous**
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28 **observation that CD81 and occludin comprise the minimal human factors required to**
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30 **render mouse cells permissive to HCV entry in vitro, we attempted murine**
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32 **humanization via a genetic approach. Here we show that expression of two human**
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34 **genes is sufficient to allow HCV infection of fully immunocompetent inbred mice. We**
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36 **establish a precedent for applying mouse genetics to dissect viral entry and validate**
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38 **the role of scavenger receptor type B class I for HCV uptake. We demonstrate that**
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40 **HCV can be blocked by passive immunization, as well as showing that a recombinant**
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42 **vaccinia virus vector induces humoral immunity and confers partial protection against**
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44 **heterologous challenge. This system recapitulates a portion of the HCV life cycle in an**
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46 **immunocompetent rodent for the first time, opening opportunities for studying viral**
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48 **pathogenesis and immunity and comprising an effective platform for testing HCV**
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50 **entry inhibitors in vivo (abstract reprinted with permission of the publisher).**
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Comment

Hepatitis C virus (HCV) infection represents a major public health burden. While only a fraction of infected individuals is able to clear acute HCV infection spontaneously, the majority of HCV-infected patients develops chronic infection. A vaccine to prevent HCV infection is not available and current therapeutic strategies are costly, limited by side effects. Thus, there is an important unmet medical need for the development of novel preventive and therapeutic strategies against HCV infection.

Research on HCV and development of antivirals has long been hampered by the lack of a small animal model and robust cell culture systems. The limited tropism of HCV for human and chimpanzee hepatocytes and its poor ability to replicate *in vitro* prompted scientists to seek for alternative models allowing deciphering parts of the viral life cycle. Major breakthroughs were the development of replicons, which for the first time permitted studying robust HCV replication in the human hepatoma cell line Huh7, and of HCV pseudo-particles (HCVpp) enabling researchers to characterize the first steps of viral entry and to assess viral neutralization (reviewed in Murray and Rice¹). The isolation of a defined genotype 2a isolate (JFH1), presenting unique *in vitro* growth characteristics, and the generation of highly HCV replication-permissive Huh7-derived cell lines finally lead to the first cell culture system producing infectious viral particles (HCVcc) (reviewed in Murray and Rice¹). Attempts to generate robust full-length clones for other genotypes did not succeed in reaching the efficiency of the JFH1 clone. The HCVcc model could however be extended to other genotypes by developing intergenotypic chimeras.^{2, 3}

The possibility to transplant primary human hepatocytes into transgenic immunodeficient mice with hepatocyte lethal phenotype (uPA-SCID and Fah/Rag2/IL-2r^{null} mice) and to subsequently infect these human hepatocytes yielded the first small animal models to study HCV infection *in vivo*.^{4, 5} Drawbacks of these models are however their high

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3 mortality, variable robustness of infection and the absence of a functional immune system
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5 that precludes the evaluation of HCV-specific immune responses and immune-based
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7 therapies or vaccines (for review see reference⁶). The development of an immunocompetent
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9 small animal model for the study of HCV infection may be achieved by two approaches:
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11 either by transplanting both human liver and immune cells in immunodeficient mice or by
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13 generating transgenic mice expressing human-specific factors to overcome the restriction of
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15 HCV infection of mouse cells. Addressing the first strategy, the reconstitution of a human
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17 immune system in human hepatocyte progenitor cell-transplanted immunodeficient Rag2^{-/-}
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19 /IL2r^{null} Balb/c mice led to the detection of human immune cells (natural killer, dendritic and T
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21 cells, but no B cells) with the low-level HCV RNA detected in the liver.⁷ Aiming to develop an
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23 immunocompetent transgenic mouse model, a proof-of-concept for the second approach to
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25 render mice permissive for HCV has been reported recently by Marcus Dorner and
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27 Alexander Ploss from the Rice laboratory at the Center for the Study of Hepatitis C,
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29 Rockefeller University, New York City.⁸
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36 Building on their previous report that mouse cells may be rendered permissive for
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38 HCV entry *in vitro* upon expression of human orthologues of the HCV entry factors SR-BI,
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40 CLDN1, CD81 and OCLN - the latter two representing the species-specific factors⁹ - Dorner
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42 and colleagues first expressed the four human HCV entry factors in mice using recombinant
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44 adenoviruses.⁸ While approximately 18-25% of murine hepatocytes coexpressed human
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46 CD81 and OCLN and 5% of the cells coexpressed the four human HCV entry factors *in vivo*,
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48 only transient low-level HCV RNA could be detected in both the serum and the liver of
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50 infected animals and no significant bioluminescent signals were obtained after infection of
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52 mice with luciferase-reporter HCV. This may partly be due to the restriction of HCV
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54 replication and assembly in mouse hepatocytes and/or from strong murine innate antiviral
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56 immune responses. This low level infection thus hampers the study of HCV infection in these
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58 humanized mice. To overcome this hurdle, the authors next developed more sensitive
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60 reporter model systems in order to monitor single-cycle HCV infection *in vivo*. Bicistronic

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3 HCV genomes expressing CRE recombinase activating either a loxP-flanked luciferase
4 reporter or a nuclear-localized green fluorescent protein (GFP)/ β -galactosidase reporter in
5 transgenic mice allowed study of single-cycle HCVcc infection in mouse hepatocytes by
6 assessing live animal bioluminescent signals or by quantifying GFP⁺ hepatocytes using flow
7 cytometry after isolation of murine hepatocytes (Figure 1).⁸ Noteworthy, expression of CD81
8 and CLDN1 mutants known to reduce HCV infection *in vitro* as well as absence of SR-BI
9 expression also reduced HCV infection of murine hepatocytes *in vivo*. These data suggest
10 that the HCV entry process *in vivo* resembles the pathway uncovered *in vitro*. Furthermore,
11 the impact of this mouse model for the study of entry inhibitors and vaccines could be
12 elegantly demonstrated by assessing the efficiency of anti-CD81 and anti-E2 antibodies in
13 reducing HCV infection. Noteworthy, immunization of mice with a recombinant vaccinia virus
14 vector expressing HCV proteins lead to robust anti-E2 antibody titers and decreased
15 susceptibility to heterologous HCV challenge. Finally, the mice were susceptible to infection
16 with recombinant viruses expressing structural proteins from genotypes 1b, 2a, 4a, 6a and
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38 In conclusion, this sensitive genetically humanized mouse model allows for the first
39 time the study of virus-host interactions during the early steps of HCV infection in the
40 presence of a functional immune system in a small animal. Thus, this model will be very
41 useful for the *in vivo* investigation of HCV entry and antibody-mediated neutralization as well
42 as the preclinical development of entry inhibitors and B cell vaccines. The preclinical
43 evaluation of entry inhibitors is of particular interest for the prevention of liver graft
44 infection.^{10, 11}. However, the lack of robust HCV replication in mouse hepatocytes and the
45 need for CRE-expressing recombinant HCVcc currently precludes the study of infection
46 using patient-derived HCV and the investigation of virus-host interactions during replication
47 and particle production.
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3 Nevertheless, despite these limitations, this study is clearly a breakthrough towards
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5 the development of an immunocompetent HCV mouse model since it opens the door for a
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7 genetically humanized mouse model for the study of the complete life cycle of HCV infection.
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9 Since a recent study has shown that mouse hepatoma cell lines are able to produce
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11 infectious virions following expression of mouse or human apolipoprotein E,¹² the
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13 identification of species-specific restriction factors for viral replication will be most likely the
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15 final step required to produce humanized transgenic mice recapitulating the entire viral life
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18 cycle.
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For Peer Review

References

1. Murray CL, Rice CM. Turning Hepatitis C Virus into a Real Virus. *Annu Rev Microbiol* 2011.
2. Pietschmann T, Kaul A, Koutsoudakis G, Shavinskaya A, Kallis S, Steinmann E, Abid K, et al. Construction and characterization of infectious intragenotypic and intergenotypic hepatitis C virus chimeras. *Proc Natl Acad Sci U S A* 2006;103:7408-7413.
3. Scheel TK, Gottwein JM, Mikkelsen LS, Jensen TB, Bukh J. Recombinant HCV variants with NS5A from genotypes 1-7 have different sensitivities to an NS5A inhibitor but not interferon-alpha. *Gastroenterology* 2011;140:1032-1042.
4. Mercer DF, Schiller DE, Elliott JF, Douglas DN, Hao C, Rinfret A, Addison WR, et al. Hepatitis C virus replication in mice with chimeric human livers. *Nat. Med.* 2001;7:927-933.
5. Bissig KD, Wieland SF, Tran P, Isogawa M, Le TT, Chisari FV, Verma IM. Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment. *J Clin Invest* 2010;120:924-930.
6. Barth H, Robinet E, Liang TJ, Baumert TF. Mouse models for the study of HCV infection and virus-host interactions. *J Hepatol* 2008;49:134-142.
7. Washburn ML, Bility MT, Zhang L, Kovalev GI, Buntzman A, Frelinger JA, Barry W, et al. A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. *Gastroenterology* 2011;140:1334-1344.
8. Dorner M, Horwitz JA, Robbins JB, Barry WT, Feng Q, Mu K, Jones CT, et al. A genetically humanized mouse model for hepatitis C virus infection. *Nature* 2011;474:208-211.
9. Ploss A, Evans MJ, Gaysinskaya VA, Panis M, You H, de Jong YP, Rice CM. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009;457:882-886.

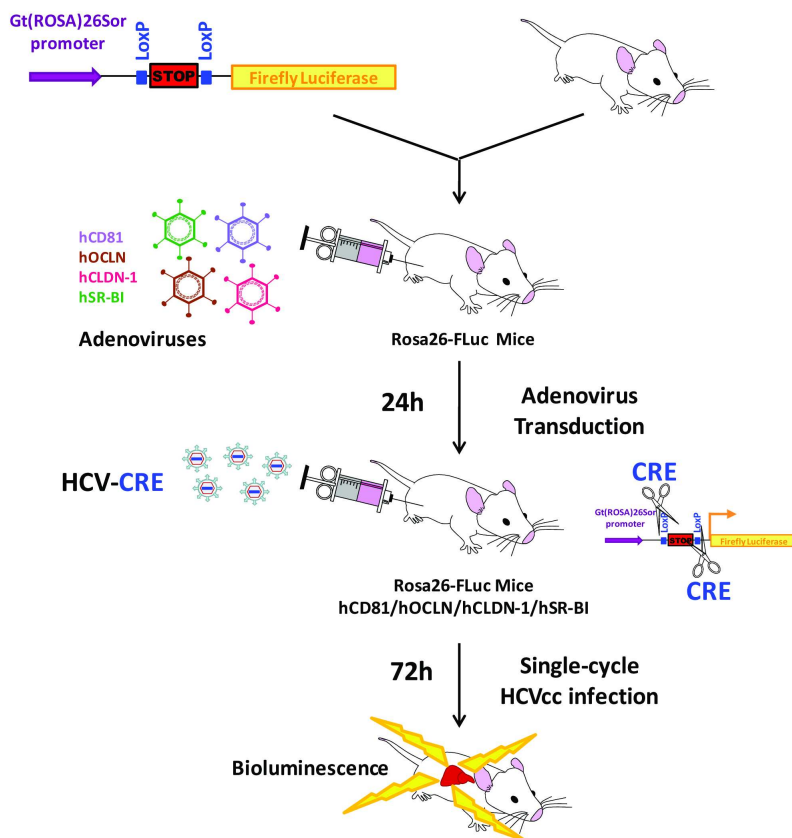
- 1
2
3 10. Zeisel MB, Fofana I, Fafi-Kremer S, Baumert TF. Hepatitis C virus entry into
4
5 hepatocytes: Molecular mechanisms and targets for antiviral therapies. J Hepatol
6
7 2011;54:566-576.
8
- 9
10 11. Fafi-Kremer S, Fofana I, Soulier E, Carolla P, Meuleman P, Leroux-Roels G, Patel
11
12 AH, et al. Viral entry and escape from antibody-mediated neutralization influence hepatitis C
13
14 virus reinfection in liver transplantation. J Exp Med 2010;207:2019-2031.
15
- 16 12. Long G, Hiet MS, Windisch MP, Lee JY, Lohmann V, Bartenschlager R. Mouse
17
18 hepatic cells support assembly of infectious hepatitis C virus particles. Gastroenterology. Jun
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Figure legend

Figure 1: Immunocompetent mouse model to study single-cycle hepatitis C virus (HCV) infection described by Dorner et al.⁸ Gt(ROSA)26Sor^{tm1(Luc)Kaelin} mice (Rosa26-Fluc) contain in the Gt(ROSA)26Sor Locus a LoxP-flanked STOP cassette restricting firefly luciferase expression. Adenoviruses encoding human CD81, OCLN, CLDN-1 and SR-BI were injected intravenously into Gt(ROSA)26Sor^{tm1(Luc)Kaelin} mice 24 h prior to intravenous challenge with recombinant bicistronic HCVcc expressing CRE protein (HCV-CRE). Upon HCV entry into mouse hepatocytes, CRE recombinase activates the luciferase reporter leading to bioluminescence signals that can be monitored using a live animal bioluminescence imaging device. In an alternative approach, CRE expression leads to activation of a nuclear-localized GFP/ β -galactosidase reporter (Rosa26-GNZ) allowing to assess HCV permissivity of murine hepatocytes by quantifying GFP⁺ murine hepatocytes using flow cytometry.

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Figure 1



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