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RESEARCH HIGHLIGHT

A new HCV mouse model on the block

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The investigation of virus-induced liver disease and hepatocellular carcinoma needs small animal models modeling hepatitis C virus (HCV) infection and liver disease biology. A recent study published in *Cell Research* reports a novel mouse model which is permissive for chronic HCV infection and shows chronic liver injury including inflammation, steatosis and fibrosis.

Chronic hepatitis C virus (HCV) infection is a major cause of liver disease worldwide. The development of direct-acting antivirals has revolutionized treatment by offering cure [1]. However, several hurdles remain. High costs limit treatment access in the majority of patients. Infection is often diagnosed at a late stage when advanced liver disease and cancer are established. Cure in advanced liver disease does not eliminate the risk of hepatocellular carcinoma (HCC). Re-infection remains possible and a vaccine is not available [2].

To better understand the pathogenesis of virus-induced liver disease and HCC, a small animal model permissive for HCV infection and modeling liver disease biology is needed [3]. HCV infection is limited to humans and chimpanzees, predominantly due to distinct host-dependency factors and innate antiviral immune responses precluding cross-infection of other species [4]. Research efforts have focused on humanizing mice permissive to HCV infection. This has led to the development of conceptually three different types of mouse models.

The human liver chimeric mouse is based on immune- and hepato-deficient mice repopulated with human hepatocytes. While the uPA-SCID [5] and FRG [6] models are extremely useful to study the viral life cycle and antivirals, the lack of an adaptive immune system and liver disease precludes the use for the study of liver disease biology and vaccine evaluation (Table 1). In the AFC8-huHSC/Hep model [7] based on modified *Rag-2*$^{-/-}$ mice, activation of the overexpressed FK506-binding protein and caspase-8 fusion protein in the liver induces death of mouse hepatocytes and facilitates engraftment of human hepatocyte progenitor and CD34^{+} haematopoetic stem cells. While infected mice exhibit liver inflammation and fibrosis, this model appears to be limited with detection of virus only in the liver (Table 1). Later, immunocompetent transgenic mice expressing the four main human entry factors (4hEF) namely CD81, scavenger receptor BI (SR-BI), claudin 1 (CLDN1) and OCLN were developed [8]. Sustained and robust HCV infection for 90 days was achieved by crossing the 4hEF mice with mice knocked out for STAT1 [9]. Furthermore, HCV infection in these mice elicited antiviral cellular and humoral immune responses. Although the animals were not reported to develop liver disease, this robust model represents a major breakthrough since it allows for studying HCV-induced immune responses and the preclinical evaluation of vaccine candidates in a small animal model (Table 1).

Complementing these achievements, a recent study published in *Cell Research* by Chen *et al*. [10] reports an immunocompetent animal model permissive for HCV infection and evidence for liver disease (Table 1). The authors describe the creation of transgenic mice expressing human occludin (OCLN) and CD81 in an outbred ICR (CD-1) background(C/OTg). The mice were permissive to low-level infection with serum- and cell culture-derived HCV and maintained stable level of HCV RNA in serum and in the liver for over 12 months. In situ hybridization of HCV RNA and staining of HCV NS5A protein in the liver cells of mice at different time points confirmed sustained infection. Moreover, an HCV protease inhibitor cured the infection. The production of infectious viruses was further confirmed by successful infection of Huh7.5.1 cells with mouse sera and successful passage of HCV into naïve C/OTg mice.

Albeit liver function remained normal (as indicated by unchanged ALT levels), the authors observed moderate hepatic inflammation in some of the persistently infected C/OTg mice as scored by Knodell histological activities in H&E staining. Moreover, both H&E and Oil Red staining showed that micro- or macro-vesicular steatotic areas increased in infected mice. Three persistently infected animals showed evidence for fibrosis and one animal exhibited early stage of cirrhosis. Evidence for neoplastic lesions was not observed [10].

In the previous report by Dorner *et al*. [9], overexpressing human CD81 and OCLN in mice with STAT1 deficiency demonstrated sustained HCV infection for ~90 days as against 12 months with ICR mice without obvious immune deficiency. To better understand the mechanisms for persistent infection in the new model, the C/OTg mice were backcrossed to C57BL/6 background

Table 1 Mouse models permissive for HCV infection.

	Human liver chimeric uPA/SCID, FRG	AFC8-huHSC/Hep	Humanized transgenic Rosa26-Fluc	C/O^{Tg}
References	[5, 6]	[7]	[8, 9]	[10]
Strain background	BalbC	BalbC	C57BL/6	ICR
Concept	Immuno- and hepatodeficient mice repopulated with human hepatocytes	Immuno- and hepatodeficient mice repopulated with human progenitor cells	Humanized for CD81, SR-BI, CLDN1 and OCLN; deficient in STAT1	Humanized for CD81 and OCLN; Modified host-dependency factor and ISG expression
Inoculum	Serum, HCVcc	Serum	HCVcc	Serum, HCVcc
Chronic infection	> 6 months	3 months	3 months	> 12 months
Viral load: serum (copies/ml)	$10^6 - 10^7$	Not reported	$10^4 - 10^5$	$10^2 - 10^4$
Viral load: liver	~10^6 *	$10^4 - 10^5$ *	$10^2 - 10^3$ *	$10^3 - 10^4$ **
Adaptive immune system	Absent	Human	Mouse	Mouse
Anti-HCV B cell responses	Absent	Not reported	Yes	Not reported
Anti-HCV T cell responses	Absent	Yes	Yes	Not reported
Evidence for HCV associated human liver disease	Absent	Inflammation, fibrosis	Not reported	Inflammation, steatosis, fibrosis

Characteristics of HCV infection, adaptive immune responses and occurrence of liver disease in HCV-permissive mouse models are listed. SR-BI, scavenger receptor class B type I; CLDN1, claudin-1; OCLN, occludin; HCVcc, cell culture-derived HCV. *copies/μg total RNA; **copies/ mg liver tissue)

to yield B6-C/OTg mice. Surprisingly, the B6-C/OTg mice did not support sustained HCV infection, indicating a potential functional role of genetic background in establishing chronic HCV infection [10]. Further investigations revealed significantly higher levels of apoE expression and progressive increase in miR-122 levels during the course of infection in C/OTg mice as compared to B6-C/OTg. In addition, the C/OTg mice showed transiently downregulated expression of anti-HCV interferon-stimulated genes (ISGs), namely *ifi44* and *Eif2ak2*, unlike B6-C/OTg mice, in the first 2 weeks post infection. Furthermore, using transgenic technology the authors demonstrated that co-expression of both OCLN and CD81 was required for susceptibility to HCV infection. Based on these results, the authors conclude that the altered expression of defined host-dependency factors combined with different innate immune responses against HCV infection facilitates the establishment of HCV infection in this particular host background.

Taken together, this study provides a novel immunocompetent HCV mouse model with evidence for HCV-associated liver diseases. The observation of liver disease in infected animals is interesting and of significant impact since it may allow the study of virus-induced liver injury including inflammation, steatosis and fibrosis — an urgent unmet need in the field. Further studies are needed to study the causal relationship between HCV, inflammation and antiviral immune responses and liver disease in this model. A potential challenge could be the lower viral load compared to other models and human blood — adaptation of viral strains to this model or further engineering of host-dependency factor expression in the mouse liver could overcome this limitation. Finally, a detailed characterization of antiviral immune responses may help to study whether this model will also be useful for vaccine development — another challenge in HCV translational research.

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