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RESEARCH HIGHLIGHT

Hepatitis C: A mouse at the end of the tunnel

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Since its discovery in 1989, researchers strive after a small animal model for Hepatitis C virus infection, so far with very limited success. A study recently published in *Nature* now for the first time reports the recapitulation of the complete life cycle of this virus in inbred mice with a functional adaptive immune system.

Worldwide, over 130 million people are chronically infected with Hepatitis C virus (HCV). Acute infection goes along with mild and generalized symptoms, and therefore mostly remains undiagnosed; in more than half of the patients, however, the infection persists and, over the years, can cause liver damage such as fibrosis, cirrhosis or hepatocellular carcinoma.

HCV is a positive strand RNA virus of the family *Flaviviridae*. Studies of this virus have made huge leaps forward since the implementation of efficient cell culture systems [1, 2]; nonetheless, our knowledge of HCV-associated pathogenesis is scarce owing to the lack of a practicable animal model. So far, chimpanzees are the only animals fully susceptible to HCV infection; however, legal, ethical, economical let alone practical reasons dictate the establishment of small animal models as an alternative. Most efforts have been put into various mouse models [3], but in general, mice are resistant to HCV infection. Only individual steps of the lifecycle could be reproduced in mouse cells: expression of human variants of the entry receptors CD81 and occludin mediates virus uptake into mouse cells [4, 5]; selectable replicons demonstrated that HCV RNA can be replicated in mouse cells, albeit inefficiently; and assembly as well as secretion of HCV particles can be achieved in mouse hepatocytes [3]. In contrast, for studying the full replication cycle in living animals, systems had to be developed, in which mouse hepatic tissue was inducibly or constitutively deteriorated to allow repopulation by human hepatocytes [3]. This interspecies chimerism naturally required the animals to be immuno-deficient to avoid graft rejection. Nonetheless, particularly the uPA-SCID model has become very popular, especially for pre-clinical drug testing and validation and for studying passive immunization strategies against HCV infection. Still, for research on vaccine development and pathogenesis, these models are of limited to no use, as such studies require robust immune responses.

The teams of Alexander Ploss and Charles Rice now report a breakthrough on the way to an immune-competent mouse model [6]. They had shown previously that adenoviral expression of human CD81 and occludin in mouse liver cells rendered fully immune-competent mice susceptible to HCV infection; however, replication was abortive [5]. In their present study, the authors take this approach two steps further: (1) by establishing mice that express the entry factors transgenically; (2) by additionally incapacitating the innate antiviral response. It had been suggested earlier that this response severely impacts HCV replication in mouse cells [7, 8], and indeed, its blocking in an otherwise fully immune-competent background sufficed to increase viral replication to detectable levels in entry factor transgenic (EFT) mice. By testing knock-outs of several factors involved in innate antiviral defense, *STAT1*$^{-/-}$ EFT mice were found to be best to support HCV replication, which was sustained for up to 11 weeks, with viral genomes detectable in both liver tissue and serum. The authors could corroborate that this viremia relied on authentic viral replication, as it could be inhibited by neutralizing antibodies or an HCV-specific antiviral compound; moreover, a drastic reduction in viral replication was observed in mice that additionally carried a knock-out of the *ppia* (Cyclophilin A) gene, a well-known HCV host dependency factor in humans [9]. On the down side, one has to acknowledge that the infection rate in the liver was stunningly low with only 0.4% of hepatocytes infected at a given time. In contrast, in human livers HCV antigen has been detected in around 20% of cells on average. This low rate of infection in mice is coherent with comparatively low infectivity titers of < 100 infectious units per ml mouse serum; somewhat in contrast, however, are the high RNA titers of 10^4-10^6 copies per ml, arguing for a high excess of non-infectious RNA-containing structures. The mechanism by which they are released into the serum of infected mice and their biophysical properties remain to be determined. Regardless of the rather sparse hepatic infection, strikingly, the authors found clear evidence for the mounting of an immune response, such as splenomegaly with increased relative frequencies of NK- and B-cells as well as infiltration of infected livers by $CD8^+$ T-cells. HCV infection was apparently cleared by the T-cell response, which at late stages of infection shifted towards a memory phenotype.

These results are remarkable and highlight the perspective of such an animal model possibly also for studies on (immune-mediated) pathogenesis and eventually development of T-cell activating vaccines. A few caveats, however, still need to be overcome on this front: none of the animals in the study developed a lasting, chronic infection, but rather cleared the virus after about 80 days; this might be resolved by adaptation of the virus to its new host by prolonged, serial passaging in mice. Additionally, the used transgenic mice lack STAT1, which is not only responsible for the immediate intrinsic antiviral response, but also required for signaling in response to all types of interferons (I, II and III). This deficiency for one deprives $CD8^+$ T-cells of their antiviral activity through interferon-γ secretion, which has been reported to be more important in controlling HCV than direct cytolytic effects [10], and, secondly, is likely to also affect the phenotypic differentiation and activation of various immune cells. One possible way to overcome the latter issue could be to use a tissue-specific knock-out of the *stat1* gene, specifically targeting hepatocytes. Alternatively, a combined knock-out of different interferon effector genes with antiviral activity against HCV might still allow for HCV replication, while restoring general responsiveness to interferons.

Even with the mentioned limitations, the presented strategy denotes a milestone for HCV research and is the first system that can truly be called a small animal model for HCV infection. As it does not rely on xenografts, it is not only more practicable and much cheaper than previous models, but also more robust as it is completely independent from variations of human graft donors. Albeit still in need for optimizations, finally one can see the long-awaited HCV-susceptible mouse at the end of the tunnel.

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References

1 Lohmann V, Körner F, Koch J, *et al*. *Science* 1999; **285**:110-113.
2 Wakita T, Pietschmann T, Kato T, *et al*. *Nat Med* 2005; **11**:791-796.
3 Mailly L, Robinet E, Meuleman P, *et al*. *Front Microbiol* 2013; **4**:213.
4 Ploss A, Evans MJ, Gaysinskaya VA, *et al*. *Nature* 2009; **457**:882-886.
5 Dorner M, Horwitz JA, Robbins JB, *et al*. *Nature* 2011; **474**:208-211.
6 Dorner M, Horwitz JA, Donovan BM, *et al*. *Nature* 2013; **501**:237-241.
7 Lin LT, Noyce RS, Pham TN, *et al*. *J Virol* 2010; **84**:9170-9180.
8 Chang KS, Cai Z, Zhang C, *et al*. *J Virol* 2006; **80**:7364-7374.
9 Lohmann V. *Curr Top Microbiol Immunol* 2013; **369**:167-198.
10 Jo J, Aichele U, Kersting N, *et al*. *Gastroenterology* 2009; **136**:1391-1401.