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Immunocompetent mouse models to evaluate intrahepatic T cell responses to HCV vaccines

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Despite considerable progress in the development of immunocompetent mouse models using different high end technologies, most available small animal models for HCV study are unsuitable for challenge experiments, which are vital for vaccine development, as they fail to measure the T cell response in liver. A recently developed intra-hepatic challenge model results in HCV antigen expression in mouse hepatocytes and through the detection of the surrogate marker, SEAP, in serum, the effect of prior vaccination can be monitored longitudinally.

Keywords: C virus, Hepatitis HCV Vaccine, mouse challenge models

Abbreviations: SEAP, secreted alkaline phosphatase; uPA, urokinase-type plasminogen activator; SCID, severe combined immunodeficiency; Rag 2, Recombination activating gene 2

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Introduction

The development of an HCV vaccine is impeded due to lack of a suitable animal model. To date, there is no convenient small animal model to study the entire life cycle of the virus. Furthermore, studies on various aspects of HCV immunity and pathogenesis are limited in those models. The chimpanzee is the most authentic model but is unavailable due to ethical reasons and expense.[1,6] Another non-rodent animal model proposed at one time was the Tree shrew (*Tupaia belangeri)*. These animals are native to China and Southeast Asia and can support HCV replication, but have not found widespread use due to low and variable infection rates and low HCV titers. The development of persistent infection and evidence for chronic liver disease are, however, advantageous features of this model. The existing animal models[5] for HCV study can be largely divided into 2 broad categories-immunosuppressed and immunocompetent models. Very recently, an attractive alternative novel intrahepatic challenge model has been developed as a new addition to the immunocompetent group.

Immunosuppressed Models

To introduce HCV into a small animal, the subject usually needs to be engrafted with human liver tissue or transplanted with human hepatocytes. To achieve this, the animal has to be immunosuppressed or immune-incompetent. The first small animal model used to study HCV was developed in 2001,[22] based on urokinase-type plasminogen activator (uPA-SCID) mice which over express urokinase, causing necrosis of murine hepatocytes that provides a significant survival advantage to transplanted human hepatocytes. Other models developed include Trimera mice (lethally irradiated mice radioprotected by transplantation of scid-mouse bone-marrow cells)[17] or Rag2 knockout mice,[4] or immunotolerized rats by perinatal exposure to human hepatocytes.[29] These models support HCV replication, but they are not immunocompetent or there is a mismatch between the rat immune system and human major histocompatibility complex (MHC) antigens on the transplanted human hepatocytes.[29] For these reasons, the immunosuppressed models cannot be used for vaccine studies.

Immunocompetent Models

In 2011, an immunocompetent model was proposed that was nevertheless based

on immunodeficient Rag2−/− γC-null mice,[28] but the immune system was reconstituted by transplantation of human haematopoietic stem cells (HSC). Cell death induced by liver-specific expression of fusion protein FK506 and Caspase 8 advantaged the co-transplantation of human hepatocyte progenitors which led to engraftment by human hepatocytes. This model was probably the first small animal model of HCV infection that could be used to study adaptive immunity and pathogenesis. After infection, HCV RNA was detected in the livers of about 50% of the mice but not in blood. This model was not used in vaccine studies probably because it is restricted by limited viral replication.

Another immunocompetent mouse model was achieved by adenovirus transduction of the mouse liver resulting in expression of 2 human cellular receptors for HCV (human CD81 and OCLN) in hepatocytes of Rosa26-Fluc mice.[9] In this model, a bicistronic HCV genome encoding CRE recombinase (HCV-CRE), which activates a loxP-flanked luciferase reporter in the mice was used. A reporter signal in the liver indicates intracellular expression of the HCV genome. The model was later modified to generate transgenic mice stably expressing the 4 human HCV entry factors.[8] Further attenuation of the host innate immunity resulted in measurable viremia over several weeks. Infected mice produced infectious HCV particles *de novo*, which could be used for antibody-related studies including vaccine-induced neutralizing antibody. This model shows great promise, but at this stage it is not practically useful in challenge experiments to examine HCV-specific cell mediated immunity.

Although, other established challenge models can be used for studies of cell mediated immunity these models fail to evaluate trafficking of T cells to the liver, the major site of HCV replication.[10,11,13]

Recent studies by Kumar *et al.* have shown that a unique β turn sequence of the human La protein if inserted into the mouse La protein, facilitates replication of the HCV genome in mouse cell lines (NIH3T3 and H6).[19] In general HCV replication is not supported in the mouse even if the viral RNA is introduced *in vivo*. However, in the presence of the humanized form of mouse La, HCV replication is more efficient. This observation raises a possibility of creating an immunocompetent HCV mouse model using human specific cell entry factors and a humanized form of La protein, which could be more effective for vaccine challenge.

The Intra-hepatic Challenge Model

Recently, Yu *et al.* developed a novel challenge model by specifically delivering HCV genes linked to the gene for the reporter molecule, secreted alkaline phosphatase (SEAP), to the mouse liver by hydrodynamic injection.[30] This model has a number of merits. The HCV antigen/SEAP is specifically expressed in mouse hepatocytes as expression of the gene is controlled by the mouse albumin enhancer and promoter. As a result, the model is able to measure the effect of the immune response in the liver after prior vaccination, especially the activity of intrahepatic effector T cells. The novel design, to co-express SEAP with the HCV antigen, allows convenient monitoring of SEAP in mouse serum as a surrogate marker for detection of intrahepatic HCV antigen, and due to the short half live of SEAP in serum, this reflects real time expression of the HCV antigen. Even when NS3 expression was too low to be detected by immunohistochemical staining or immunoblotting, SEAP activity was still detectable in the serum, showing that SEAP detection is more sensitive than detection of the HCV antigen in liver. The detection of SEAP also allows longitudinal analysis of the dynamics of immune response without culling individual mice and the model represents for the first time a mouse that can be used as an intra-hepatic challenge model for HCV vaccine studies.

Possible Expansion of Existing Challenge Models

The above model developed by Yu *et al* is convenient and cheap, and the ability for longitudinal analyses represents a major advantage. Furthermore, the model is very versatile as any HCV antigen/SEAP combination can be expressed. It will also be possible to use HLA-A2.1 transgenic mice to study human HLA restricted T cell responses and identify specific T cell epitopes.[3,23,25,26] A comparison of the immunogenicity of different HCV proteins and vaccines in wild type and transgenic mice could yield important information on candidate HCV vaccines, in terms of antigen optimization, adjuvant selection and specific host responses.

Inclusion of the HCV 4A gene in DNA vaccine constructs is debatable, since the NS3/4A protease cleaves MAVS (IPS-1) which may attenuate the innate immune response and therefore influence the resultant adaptive immune response,[15,21] while it has been suggested that the NS4A protein stabilizes the NS3 protein resulting in increased immunity.[12] The model proposed by Yu et al can determine any advantage of the use of NS3 rather than NS3/4A. Other studies also showed that additional HCV proteins have roles to evade or suppress innate immunity. The core protein inactivates IFN signaling through regulating the JAK-STAT pathway,[2,16,20] NS5A and E2 regulate the PKR pathway.[14,27] NS4B interacts with STING[7,24] and NS2 inhibits IKKε and TBK1 functions.[18] Informed decisions on whether to include these antigens in vaccine constructs or to abrogate the immunosuppressive function of the antigen by mutation while retaining its immunogenicity can be addressed by this model.

Conclusion

The holy grail of a HCV animal model is to capture the complete life cycle of HCV and support high level virus replication in an immunocompetent small animal which can be used for drug and immunological studies. The humanized model is promising but there are still a number of obstacles to overcome. The intrahepatic challenge model uses well defined technology which has acquired a new life by including the SEAP reporter

gene. The use of this animal model may help define the optimum strategy to induce effective T cell response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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