HCV's mighty mouse

By Tracey Baas, Senior Editor

The most widely used animal model of HCV infection requires transplanting human hepatocytes into immunodeficient mice. These mice are useful for testing antivirals, but the absence of an immune system makes the animals unsuitable for studying other classes of HCV products such as vaccines and immunotherapeutics. Now, researchers from **The Rockefeller University** have developed an immunocompetent, genetically humanized mouse that allows limited HCV infection, albeit not replication.¹

The researchers are in discussions with an undisclosed company interested in exclusively licensing the technology to evaluate HCV entry inhibitors and vaccines.

The Rockefeller group used a two-pronged approach to develop the model: genetically humanizing the mice with human HCV entry

factors and infecting the animals with a sensitive HCV luciferase reporter system.

First, the researchers intravenously delivered adenovirus vectors encoding human host entry factors CD81, scavenger receptor class B member 1 (SCARB1), claudin 1 (CLDN1) and/or occludin (OCLN) to mice. About 18%–25% of murine hepatocytes had strong expression of human CD81 and OCLN, and all 4 proteins showed up in about 5% of murine hepatocytes.

To detect HCV infection in the liver, mice were engineered to express the *luciferase* gene following inoculation with HCV.

The signal peaked at 72 hours postinfection and subsequently decreased, suggesting a single cycle of HCV infection occurred without additional viral replication. This limits the system's use to screening inhibitors of HCV entry but not inhibitors of other aspects of the HCV life cycle (see **Box 1**, “A vaccine is not just a vaccine”).

The researchers suspect the short viral life span is due to both the host immune response induced by the adenovirus vectors used to express the human host entry factors and the lack of human-specific host factors needed to foster assembly and release of infectious virus particles.

Box 1. A vaccine is not just a vaccine.

Although the conventional mouse model of HCV allows for testing of antivirals, and the new model from The Rockefeller University facilitates testing of viral entry inhibitors and some vaccines,¹ at least one class of HCV drugs—T cell vaccines—remains in need of a model system. Researchers at The University of North Carolina at Chapel Hill have developed a humanized mouse to study HCV infection, immune response and liver disease that might fit the bill.

To develop a more useful model to study HCV pathogenesis and treatment, the Chapel Hill researchers created a chimeric mouse transplanted with human hepatocytes that allows low-level HCV infection, with a complete HCV life cycle, and contains a partially reconstituted human immune system.²

In the humanized livers of mice with a partially reconstituted human immune system, low-level viral RNA and human CD4⁺ and CD8⁺ T cells, NK cells, and plasmacytoid and myeloid dendritic cells were detected. With the combination of HCV infection and human leukocyte infiltration, the humanized liver also showed inflammation and fibrosis,

allowing for characterization of HCV pathogenesis.

Also in the infected mice, HCV-specific T cells could be expanded and showed HCV-specific T cell responses after restimulation with HCV peptides, suggesting an active immune response.

The team included researchers from Rockefeller and the Chinese Academy of Sciences. The work was published in *Gastroenterology*.

Drawbacks of this xenotransplant model include low repopulation levels of human hepatocytes and an absence of antibody responses due to the lack of fully functional B cells.³

Alexander Ploss, assistant professor at Rockefeller, had a hand in developing both the xenotransplant model and the immunocompetent humanized mouse model published in *Nature*. “The immune-competent, genetically humanized mouse at this point is certainly more geared towards vaccine candidates that are primarily based on protection via antibodies,” he told *SciBX*. “The new humanized xenotransplant model has certainly a lot of promise, and this also is an area that we are heavily engaged

in. However, while some activation of the human immune system has been observed, immune functionality remains very poor. T cells are not adequately selected in these mice, lymphoid architecture required for proper priming of immune responses is suboptimal and certainly antibody isotype switching and affinity maturation have not been convincingly demonstrated.”

Lishan Su, the principal investigator for the xenograft model and a professor in the Department of Microbiology and Immunology at Chapel Hill, said there is no question that the T cell response is functional. He is hoping to improve the immune response by transplanting human thymus tissue into the model mice—a procedure developed in 1988 (ref. 4)—and to raise the level of HCV infection by increasing numbers of human hepatocytes that engraft in the mouse liver. He has already used the model to study aspects of HIV/HCV co-infection, which he will publish in the near future.

Su's mice have been patented, and licensing discussions with an undisclosed company are ongoing.

—TB

Table 1. No animal model does it all. A handful of animal models for HCV are available to researchers, who will need to examine the pros and cons to find the right one for their experiments.

Model	Origin of HCV	Host modifications	Pros	Cons
Chimpanzees	Primary patient isolates Recombinant infectious HCV molecular clones	No modifications	Antiviral immune response and pathology can be studied Necroinflammatory hepatic lesions observed Increased serum aminotransferase levels observed	Clinical course of infection is milder than that of humans Not all challenged animals become infected Liver cirrhosis or fibrosis not reported Limited by costs and ethical concerns
Mice with genetic humanization	HCV genome expressing Cre recombinase	Genomic loxP-flanked firefly luciferase reporter Adenovirus-delivered human HCV reporter entry genes	Immunocompetent mouse Shows HCV entry Can be used to evaluate B cell vaccines	Lacks full virus life cycle Hepatic pathology absent No viremia
Mice with liver humanization	Primary patient isolates Recombinant infectious HCV molecular clones	Liver repopulated with human hepatocytes	Full viral life cycle Shows viremia	Lacks immune system Technically challenging to produce but can be bought
Mice with liver humanization plus immune reconstitution	Primary patient isolates Recombinant infectious HCV molecular clones	Liver repopulated with human hepatocytes Immune system reconstituted with human hematopoietic progenitors	Partially reconstituted human immune system Liver develops inflammation and fibrosis Full viral life cycle Might be useful for evaluating T cell vaccines	Low-level viral replication No viremia Lacks fully functional B cells Technically challenging to produce

HCV infection took place in about 5% of murine hepatocytes expressing human CD81 and OCLN and in up to 20% of murine hepatocytes expressing all 4 factors, indicating that expression of CD81 and OCLN is sufficient for HCV infectivity.

The researchers said the range of infection frequencies resembles that seen in HCV-positive patient tissues. Indeed, the mouse model mimics the hepatocyte context of a chronically infected patient and would provide a reasonable entry inhibitor assessment model, said Geneviève Inchauspé, department head of infectious diseases at **Transgene S.A.**

Finally, the researchers used the new mouse model to evaluate a recombinant vaccinia virus vector expressing five HCV genotype 1a proteins. Immunized mice showed HCV antibody production and partial protection against challenge of genotypes 1b, 2a or 4a compared with unimmunized mice.

In addition to Rockefeller, the team included researchers from **The Scripps Research Institute** and the **Ragon Institute of MGH, MIT and Harvard**. Results were published in *Nature*.

Lorne Tyrrell and Thomas Baumert both said the next steps should include producing mice with hepatic knock-in of the human HCV entry factors CD81 and OCLN. Tyrrell is professor of medical microbiology and immunology at the **University of Alberta** and director of the **Li Ka Shing Institute of Virology at the University of Alberta**. Baumert is professor of medicine at the **University of Strasbourg** and head of the Strasbourg Virology Unit U748 at the **Institut National de la Santé et de la Recherche Médicale (INSERM)**.

Team leader Alexander Ploss, assistant professor at Rockefeller University, agreed and said production of such mice is under way.

Murine pros and cons

The new model will not be replacing the commonly used immuno-

deficient model because it does not recreate all phases of the virus life cycle—only viral entry. In addition, the immune responses in the new model are limited primarily to B cell–secreted neutralizing antibodies.

Baumert thinks the new model will be most attractive to researchers who want to evaluate entry inhibitors and vaccine candidates that elicit neutralizing antibodies. For the model to be more applicable to evaluating T cell–based vaccine candidates that target viral nonstructural proteins, the virus would have to undergo not only infection of a cell but maintain active viral replication and production.

“Clearly, Ploss’ model represents a powerful way to realize the first screening of some classes of HCV therapeutics but not a so-called T cell–inducing vaccine like our TG4040,” said Inchauspé.

Transgene’s TG4040 recombinant modified vaccinia Ankara (MVA) virus containing nucleotide sequences encoding the immunogenic HCV nonstructural protein 3 (NS3), NS4 and NS5B is in Phase II testing.

“The new model will definitely not replace the immunodeficient human liver chimeric mice,” noted Tyrrell. “Chimeric mice can now be produced with a much lower mortality than originally seen with the first microsurgies and can be obtained at **KMT Hepatech Inc.** or **PhoenixBio Co. Ltd.**, which licensed the model from KMT Hepatech.”

“And although the immunodeficient chimeric mice require special handling and housing to keep them in a germ-free environment, you have a mouse that can handle the full replication cycle of the virus and shows levels of virus in the plasma, similar to that seen in chronically infected HCV patients,” added Tyrrell. “You don’t get active replication in the genetically humanized mouse model. HCV infection is specific to individual hepatocytes in the liver, and the virus doesn’t replicate or spread to neighboring hepatocytes or to the blood.”

“The new mouse model provides a good complement to the xenotransplanted immunodeficient mouse model.”

**—Geneviève Inchauspé,
Transgene S.A.**

Currently, each animal model of HCV has a distinct set of limitations (see **Table 1**, “**No animal model does it all**”). Inchauspé agreed. “The new mouse model provides a good complement to the xenotransplanted immunodeficient mouse model. While the latter model allows propagation of HCV because hepatocytes are of human origin, the immune system of mice is hampered, preventing or rendering very tricky the testing of ‘active’ immune interventions such as vaccines. Ploss’ mice are easy to access and to manipulate—no specific confinement required—and the mice are cheap and above all, immunocompetent, thus opening the road for testing of ‘active’ vaccination via use of antibody-inducing vaccines. These features cannot be provided by the xenotransplanted model,” she said.

Rockefeller University has filed for a patent covering the mouse model.

Baas, T. *SciBX* 4(24); doi:10.1038/scibx.2011.675

Published online June 16, 2011

REFERENCES

1. Dorner, M. *et al. Nature*; published online June 8, 2011; doi:10.1038/nature10168
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2. Washburn, M.L. *et al. Gastroenterology* **140**, 1334–1344 (2011)
3. Robinet, E. & Baumert, T.F. *J. Hepatol.*; published online May 14, 2011; doi:10.1016/j.jhep.2011.02.038
4. McCune, J.M. *et al. Science* **241**, 1632–1639 (1988)

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