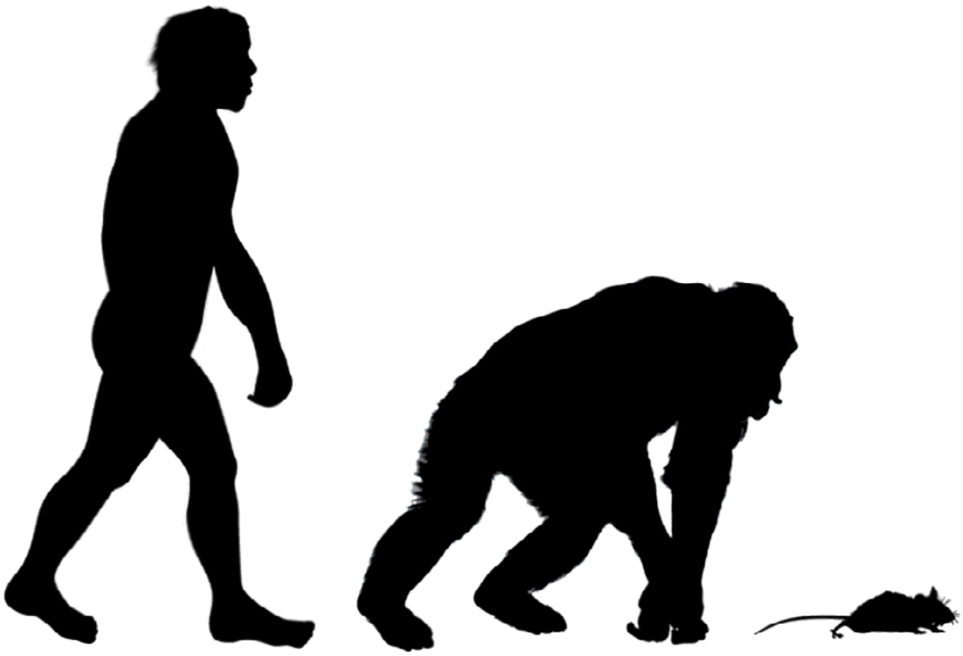

RESEARCH TECHNIQUE

The murine candidate

Small animals that mimic human hepatitis C infection will help researchers pinpoint weakness in the viral life cycle.

BY ELIE DOLGIN

The hepatitis C virus (HCV) is hard to study. Most of what researchers know about how it multiplies comes from cell-culture systems. Such cellular set-ups have proven invaluable for developing new drugs, including protease inhibitors and polymerase inhibitors, which prevent the virus from replicating its components inside the cell. Yet these cell-based systems fail to capture other important parts of the viral life cycle, such as the step before replication, when the virus attaches to liver cells and gains entry. What's more, cell-culture systems cannot reproduce the interaction between the immune system and the virus nor can they recapitulate entire organs so that liver pathology can be studied. For these reasons, researchers interested in how the virus causes disease have long sought a small-animal model.

Common laboratory animals, including rodents and most primates, are not susceptible hosts for HCV. Scientists have therefore had to settle for chimpanzees, which, like humans, are vulnerable to chronic HCV infections. However, "for ethical and economic reasons, the chimp is a terrible model," says Matthew Evans, an HCV researcher at the Mount Sinai School of Medicine in New York. Research involving chimpanzees is banned in many parts of the world, including Europe. And in most places where experimentation with great apes is allowed, laws against euthanizing chimps require investigators to fund the animals' long-term care — a prohibitively expensive commitment.

That's where a colony of ordinary-looking black mice running around in cages on the fourth floor of the Rockefeller University Comparative Bioscience Center in New York comes in. These animals might not look special, but they have been engineered to express either a pair or a quartet of human genes and, as such, are the first small animals with fully functioning immune systems that are prone to HCV infection. Using these models, "you can actually now look at hepatitis C virus entry *in vivo*," says Rockefeller immunologist Alexander Ploss, who developed the animals together with Charles Rice, executive and scientific director of the Center for the Study of Hepatitis C in New York.

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These mice, and others like them, could provide a cheaper, more robust and less ethically fraught route to HCV drug and vaccine discovery.

THE HUMAN SIDE

Getting to this point has been a hard slog. In the years immediately after the virus was first described in 1989, many research teams developed transgenic mice carrying one or more genes encoding HCV proteins. Thus it was possible to study HCV-induced liver pathology without infecting mice with the virus. This approach still has some proponents. Last year, a team led by Matti Sällberg, a viral immunologist at the Karolinska Institute in Stockholm, used mice expressing the viral protease and showed that treatment with a drug targeting the cytokine tumour-necrosis factor-α led to improved liver function[1].

But the approach is highly artificial, leading to overexpression of the introduced viral genes and ignoring the rest of the viral life cycle. Over the past decade, most researchers have moved away from this set-up in favour of systems that involve infecting animals with the virus.

The first such model was reported ten years ago by a team led by transplant surgeon Norman Kneteman at the University of Alberta, in Edmonton, Canada. Kneteman's group engineered mice to express a gene that kills off the animals' own liver cells, which aren't susceptible to HCV infection; in their place they transplanted human liver cells, which are. These mice with humanized livers could be infected with HCV[2]. "This was the first [mouse] model that actually allowed HCV infection for prolonged periods of time by the normal route," says Kneteman.

These animals have proven useful for testing many candidate drugs. For example, a Japanese team led by Hiroshima University's Kazuaki Chayama treated Kneteman's liver transplant mice with a combination of new drugs: the protease inhibitor telaprevir (from Vertex Pharmaceuticals, based in Cambridge, Massachusetts) and the experimental polymerase inhibitor MK-0608 (from drug giant Merck, headquartered in Whitehouse Station, New Jersey). Late last year, the team reported that this combination eliminated the virus from the animals after a month of therapy and prevented the emergence of drug resistance, which often arises in mice and humans treated with either drug alone[3].

But to facilitate the human tissue transplant, the mice must be engineered to lack components of their immune system. The animals are thereby rendered poor models for testing drugs that alter the immune system, known as immunotherapies. Generating these mice also presents special difficulties. For one,

researchers can't breed chimaeric animals. And the mice are sickly because of the liver toxic gene.

Two recent transplant models of HCV infection provide improvements over Kneteman's mice[4,5]. Both types of mouse are less frail because of technical workarounds that allow researchers to introduce the liver deficit later in life. The model developed by Lishan Su, an immunologist at the University of North Carolina at Chapel Hill, in collaboration with Ploss and Rice at Rockefeller, also involves transplanting human blood stem cells into the animals to reconstitute a human-like immune system[5]. Of all of the published reports, says Su, "this is the only one that has both the immune system and the human liver in a chimaeric animal", creating a living platform for testing vaccines and immunotherapies in a human-like model.

Even though Su's mice generate a human T-cell response against the virus when infected, they still lack a complete immune system. "What we need now is a mouse — an immunocompetent, normal, mouse — that can be infected by a hepatitis C virus capable of replicating, spreading and initiating an immune response," says Frank Chisari, a virologist at The Scripps Research Institute in La Jolla, California. "We are light years away from that because that virus does not like to infect or replicate in mouse cells." But scientists are getting closer.

ENTRY LEVEL POSITION

To gain entry into liver cells, HCV hijacks four proteins. Although mice naturally produce these proteins, the human versions of two of them are needed for viral entry[6]. The black rodents at Rockefeller are the first animals into which the required human entry factors have successfully been introduced. "This has a lot of applications," says Ploss. "Right now, it's useful to measure HCV entry and potential entry inhibitors."

"This is a big advance," says Michael Houghton, a virologist at the University of Alberta, who co-discovered HCV more than 20 years ago. "It's been difficult to do vaccine research for hepatitis C because of the lack of an animal model other than the chimp. Now we can start using different vaccine strategies in mice to see which are best at eliciting a protective response."

Ploss's mice are the first such animals with a fully intact immune system that are susceptible to the viral infection. But the infection stops after cell entry: the virus does not seem to replicate. "You can recapitulate HCV entry," says Ploss, "but replication is still very inefficient and not detectable by conventional methods." So the big challenge now remains identifying whether additional human factors are needed to achieve the next step of the HCV life cycle in mice.

After replication comes assembly, when the viral components are gathered into new infectious particles that will be released from the cell and invade other cells. Fortunately, this final stage in the viral life cycle seems to be possible in mouse cells without introducing any human proteins, according to research presented at this year's International Liver Congress, in Berlin, by Ralf Bartenschlager, a molecular virologist at Heidelberg University in Germany. If the barriers to replication can be overcome, Bartenschlager says, it should be straightforward to get a full infection cycle going in a mouse. "We have the early steps; we have the late steps; the big black box now is the step in between."

It took more than a decade for scientists to deduce the factors needed for HCV cell entry. But Thomas Baumert, a hepatologist and virologist at the University of Strasbourg in France, is confident that the community will solve the problem of replication much faster. "We have better model systems now, so I think we can advance more rapidly." Within five years, he predicts, "it will be possible to produce transgenic mice for the entire viral life cycle".

Rice is equally confident this approach will work — but he is hedging his bets. Even such a model would have its drawbacks, he says, because the more mouse-like the model, the further removed it is from the human system. That's why even as his lab is aggressively pursuing a transgenic animal, he maintains active collaborations to develop other models, including new transplant chimaeric mice with humanized livers and immune systems. Other researchers are looking to animals that provide the natural susceptibility of primates without the ethical baggage (see 'The turn of the shrew'). "All of these things should be pursued in parallel," Rice says, "because we really don't know which of these models is going to be the best for a given application."

And so Rice and others continue to try and build a better mouse to help the research community beat a path to new HCV treatments. ■

THE TURN OF THE SHREW

Unusual model isn't persuading researchers of its practicality

Although most of the work developing small-animal models of hepatitis C virus (HCV) infection has focused on mice, some research teams have advanced an alternative model: the northern treeshrew (Tupaia belangeri). This squirrel-shaped animal shares a common ancestor with apes and is the only non-ape species known to be naturally susceptible to HCV. Last year, the first longitudinal analysis of HCV-infected tree shrews showed that, over the course of three years, the animals developed chronic hepatitis, fatty liver degeneration and liver cirrhosis[7]. "It's very similar to HCV infection in human beings," says study co-author Kyoko Tsukiyama-Kohara of Kumamoto University in Japan.

But few research teams have managed to establish long-term infections in the animals. And given the limited track record of tree shrews in drug discovery, most scientists agree that more traditional lab animal models of infection, such as mice, are needed. "If you're going to take a multimillion dollar drug and do your final trial before you go into humans, you need to have a reproducible model," says Robert Lanford, who has studied HCV in chimps for more than 20 years at the Texas Biomedical Research Institute in San Antonio.

NEIL BOWMAN/FLPA

The northern treeshrew, a natural host to hepatitis C virus, is proving an unpopular model of infection.

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