Sarepta's hemorrhagic mouse

By Tracey Baas, Senior Editor

A team at **Sarepta Therapeutics Inc.** has developed a mouse model of arenavirus infection that recapitulates key symptoms of the disease including hemorrhagic fever and multiorgan failure.¹ The researchers now want to use the mice to study the Lassa arenavirus.

Arenavirus infection symptoms vary from virus to virus, but general symptoms are fever, malaise, body aches and rash. For arenaviruses that cause hemorrhagic disease, symptoms also include low platelet count, internal and external hemorrhage and multiorgan failure.²

Arenaviruses are divided into two serogroups: Old World and New World, which differ genetically and by geographical distribution. Historically, only the New World hemorrhagic fever arenaviruses appear to cause a set of neurologic disease symptoms.²

Arenavirus infection typically is treated with best supportive care and the generic antiviral ribavirin. Targeted therapies do not exist

and have been difficult to develop because no rodent model accurately mimics human arenavirus infection.

Sarepta's initial plan was to characterize its own morpholino-based arenavirus antiviral, dubbed AVI-7012, in mice expressing a fluorescent transgene. Those mice were derived from the FVB mouse strain, which is named for its susceptibility to the B strain of Friend leukemia virus and has enhanced susceptibility to a variety of viruses.³

When the researchers infected the FVB mice with an arenavirus called lymphocytic choriomeningitis virus 13 (LCMV-13), the animals showed signs of mucosal, cutaneous and organ hemorrhaging. That result was unexpected because LCMV is a prototype laboratory arenavirus strain that typically causes only mild infection in humans.

To confirm that the fluorescent transgene was not causing the hemorrhaging, the team repeated the experiment using FVB mice not expressing the transgene. LCMV-13 infection again caused hemorrhagic fever-like symptoms—platelet loss, cutaneous hemorrhaging and hepatic dysfunction. Seven of eight mice died six to eight days postinfection.

The researchers next used the hemorrhagic mice to test the antiviral activity of AVI-7012. The compound decreased viral load compared with saline but surprisingly did not result in decreased mortality.

In animals with T cell depletion or Il-17 receptor C (Il17rc) knockdown, LCMV-13 infection resulted in decreased viral loads, organ damage and death compared with what was seen in wild-type mice. These results suggest that arenavirus alone does not cause the pathology and that T cell-produced Il-17 may exacerbate arenaviral hemorrhagic fever-like disease in FVB mice.

Finally, the Sarepta team generated a third chimeric mouse strain by crossing the FVB mice with standard C57BL/6 lab mice. The result was a mouse strain compatible with available mouse immunological reagents and with a better correlation between increased virus replication and increased disease symptoms.

High virus infectivity resulted in 100% death, medium virus infectivity resulted in 66% death and low virus infectivity resulted in no death.

Results were reported in *PLoS Pathogens*.

More mouse, different virus

"We have ongoing work using the model in-house to explore host-virus interactions that contribute to hemorrhagic symptoms," said Dan Mourich, director of immunology at Sarepta and principal investigator on the manuscript. "Our goal is to use the chimeric mouse to study other types of arenaviruses, such as Junin or Lassa, which cause hemorrhagic fever in humans. But in order to do that, we will have to rely on collaborators that have access to laboratories with proper biosafety containment."

Lassa, an Old World strain, is the most prevalent hemorrhagic fever-inducing arenavirus found in West Africa.

Sarepta previously has worked with the **U.S. Department of Defense** to investigate antivirals in mouse models of Ebola and Marburg infection.

Juan Carlos de la Torre, professor of immunology and microbial science at **The Scripps Research Institute**, thinks the next steps should be showing that chimeric mice infected with Lassa virus (LASV) recapitulate key features of Lassa fever disease, with increased viral load correlating to increased disease symptoms, and pathology unrelated to T cell immune-mediated damage of infected cells and tissues.

"It would provide tremendous possibilities for the investigation of disease mechanisms and therapies. Current mouse models of Lassa infection are rather artificial. They lack a fully functional immune system or require the use of rather specific viral strains, which raises some issues about whether information derived from their use would accurately reflect the nature of LASV infection," he said. "It would also be very important to determine if Lassa-infected FVB chimeric mice show disease that is similar to Lassa-infected nonhuman primates. This would make compound screening in the chimeric mice more useful to translation in nonhuman primates."

De la Torre did say two issues presented in Sarepta's study of LCMV-13 may limit the use of these chimeric mice to study Lassa virus.

"First, they show that T cells play a critical role in arenavirus-induced disease pathology. This is in contrast to what is known about human patients succumbing to Lassa virus infection. Patients show very limited immune responses, immune cell infiltrates and tissue damage," de la

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Torre said. “Second, the study shows limited correlation between viral load and disease outcome. In humans, levels of virus in the blood are a good predictor of disease outcome for Lassa fever.”

“It is possible that the model does not fully reflect the human disease; however, making precise comparisons of immunological and viral endpoints in a mouse model to those observed in the clinic are difficult at best,” said Mourich. “The exact time of infection, inoculum size, peak of virus production in all tissues as well as the peak and specific nature of T cell responses in a clinically manifested disease are rarely measurable or measured.”

“In our mouse model we are stating that the level of virus dose does not directly predict disease outcome,” Mourich continued. “We would not discount that detection of virus in the blood of a human patient would not be predictive of Lassa disease severity.”

Eric Vela, senior research scientist at **Battelle Biomedical Research Center**, would like to see the group use the chimeric mouse model to evaluate if there are differences between Old World and New World arenavirus disease outcomes.

“Sarepta did not show if there was a neurological component in the chimeric mice infected with LCMV-13. Researchers have previously shown hemorrhagic symptoms and neurologic disease—tremors, and shaking and rocking behavior—in Syrian golden hamsters infected with the New World Pirital virus,” he noted.

“If neurological disease is found to occur in the FVB mice, they might be considered better suited to model New World arenavirus infections.

If neurological disease is absent, they might be considered better suited to model Old World arenavirus infections,” Vela added.

“We would hope to explore if there exists a neurological component in the mouse disease model,” Mourich said. “Observation of virus in the brain might be an initial clue that there is some disruption in the blood brain barrier either caused by the virus or as a result of the profound inflammatory damage.”

The chimeric mouse model of arenavirus infection is patented by Sarepta and is available for licensing.

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Contact: Dan V. Mourich, Sarepta Therapeutics Inc., Cambridge, Mass.
e-mail: dmourich@sareptatherapeutics.com
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COMPANIES AND INSTITUTIONS MENTIONED

Battelle Biomedical Research Center, Columbus, Ohio
Sarepta Therapeutics Inc. (NASDAQ:SRPT), Cambridge, Mass.
The Scripps Research Institute, La Jolla, Calif.
U.S. Department of Defense, Washington, D.C.