Development of a Lethal Rodent Model of Lymphocytic Choriomeningitis Virus Infection for Preclinical Antiviral Drug Testing

Rhett Fackrell[1,2], Jonna B. Westover[2], Jung-Ae Choi[2], Brian B. Gowen[2]

[1] Department of Biology, Utah State University, Logan, UT.

[2] Department of Animal, Dairy, and Veterinary Sciences and Institute for Antiviral Research, Utah State University, Logan, UT.

Abstract

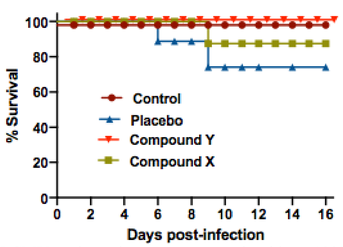
Lymphocyctic Choriomeningitis virus (LCMV) is an Old World arenavirus belonging to the family *Arenaviridae*. The natural host is the common house mouse, with about 5% of the population persistently carrying and shedding the virus. LCMV is spread to humans through the aerosolized excrements of rodents or passed from mother to fetus through the placenta. Some of these cases lead to aseptic meningitis or meningoencephalitis with permanent neurological damage possible. Studies indicate that 2-5% of people are positive for serum LCMV antibodies [1]. LCMV infection has also occurred in organ transplant recipients, with nearly all cases being fatal [2]. There are currently limited treatments for LCMV infection, including the antiviral ribavirin which has associated toxicity. This highlights the need for a suitable animal model to evaluate potential antiviral compounds. This research aims to develop a more robust and consistent lethal mouse model of LCMV infection that can be utilized to assess promising antiviral compounds. An animal disease model that is consistently lethal and produces clinical manifestations seen in the human disease will have a dramatic positive effect on identifying successful treatment. This work is especially critical for fetuses that contract the disease congenitally and immunosuppressed organ transplant recipients at the highest risk to the often fatal effects of an LCMV infection.

1. Mathur G, Yadav K, Ford B, et al. High clinical suspicion of donor-derived disease leads to timely recognition and early intervention to treat solid organ transplant-transmitted lymphocytic choriomeningitis virus. Transpl Infect Dis 2017; Aug;19(4):e12707.

2. Amman BR, Pavlin BI, Albariño CG, Comer JA, Erickson BR, Oliver JB, Sealy TK, Vincent MJ, Nichol ST, Paddock CD, Tumpey AJ, Wagoner KD, Glauer RD, Smith KA, Winpisinger KA, Parsely MS, Wyrick P, Hannafin CH, Bandy U, Zaki S, Rollin PE, Ksiazek TG. Pet rodents and fatal lymphocytic choriomeningitis in transplant patients. Emerg Infect Dis. 2007 May;13(5):719-25.

Introduction

- Without a lethal model, experimental disease signs are limited and without obvious indication of treatment success. A lethal model gives clear indication of drug success, because the infected subject will present with death in an unsuccessful treatment. Development of a lethal model is necessary for accurate observation.



Recent LCMV study testing two compounds without 100% lethality. Placebo mice survive infection. Unclear observation of compound treatment.

- A recent study obtained 100% lethality with LCMV clone 13 in the B6.PL-Thy1a/CyJ (B6.PL) mouse strain [3]. To improve success probability of our model, we chose the same mouse strain and LCMV clone.
- In late 2010, 4 organ recipients received transplants from a single donor. Every recipient experienced severe illness, and 2 died. Subsequent testing revealed the donor had been infected with LCMV resulting in their deaths [4]. Mitigating the risk of chronically infected organ donors transmitting LCMV to organ recipients is a high priority.

3. Misumi I, Cook KD, Mitchell JE, Lund MM, Vick SC, Lee RH, Uchimura T, Bergmeier W, Mieczkowski P, Pardo-Manuel de Villena F, Ting JPY, Whitmire JK. Identification of a Locus in Mice that Regulates the Collateral Damage and Lethality of Virus Infection. Cell Rep. 2019 Apr 30;27(5):1387-1396.e5.

4. Macneil, A., Ströher, U., Farnon, E., Campbell, S., Cannon, D., Paddock, C. D., Drew, C. P., Kuehnert, M., Knust, B., Gruenenfelder, R., Zaki, S. R., Rollin, P. E., Nichol, S. T., & LCMV Transplant Investigation Team (2012). Solid organ transplant-associated lymphocytic choriomeningitis, United States, 2011. *Emerging infectious diseases*, *18*(8), 1256–1262.

Objectives

- Achieve 100% lethality of LCMV clone 13 challenge in the B6.PL mouse strain.
- Collect and store homogenized tissue samples that contain passaged virus producing the lethal model.
- Future sequencing of the passaged virus strains to determine what mutations produced a lethal model.

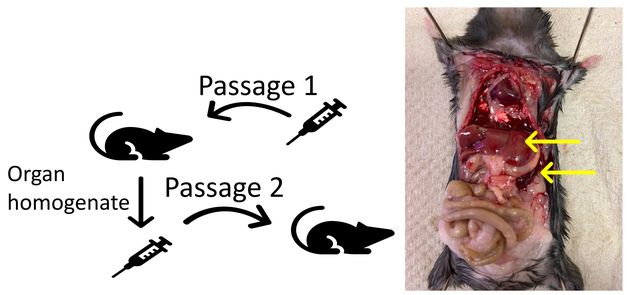
Methods

Animals: Male and female 4 week-old B6.PL and SJL/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). They were quarantined for 3 days prior to challenge and fed Harlan Lab Block and tap water ad libitum.

Ethics regulation of laboratory animals: All animal procedures used in this study complied with guidelines set by the USDA and Utah State University Animal Care and Use Committee.

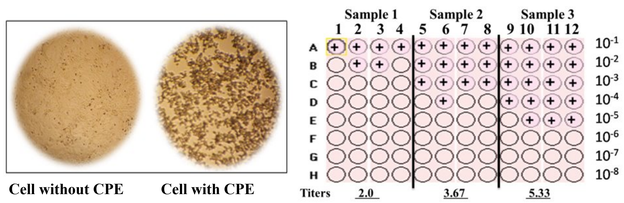
Virus: LCMV Clone 13 (a variant of the Armstrong parent strain) was provided by Dr. Maria Salvato (University of Maryland School of Medicine, Baltimore, MD). The virus used to infect mice was from a stock prepared following 1 passage in BHK-21 cells. The stock was diluted in minimal essential medium (MEM, Hyclone, Logan, UT) just prior to challenge by intraperitoneal (IP) injection of 0.1 ml (ventral).

Experimental design: Mice weighed the day of the infection and randomized to normalize weight distribution. Weights were measured daily, and animals were necropsied at their lowest weight. Whole liver and spleen organs collected during necropsy. These organs were individually homogenized in MEM and frozen for future evaluation and infection of later passages. To infect the following passages, homogenates were clarified via centrifuge, and the supernatant used for infection.



Graphical depiction of how virus passages advance (left image). Photo of a mouse necropsy (right) indicates the liver (top) and spleen (bottom) organs harvested for tissue homogenate and viral titer analyses.

Serum virus titers: Virus titers were assayed using an infectious cell culture assay. Briefly, a specific volume of serum was serially diluted and added to triplicate wells of Vero (African green monkey kidney) cell monolayers in 96-well microtiter plates. The viral cytopathic effect (CPE) was determined 7 days after plating and the 50% endpoints were calculated as described [5]. The lower limits of detection were 1.67 $\log_{10}$ 50% cell culture infectious dose ($CCID_{50}$) per ml serum, and 2.2 $\log_{10}$ $CCID_{50}$/g tissue.

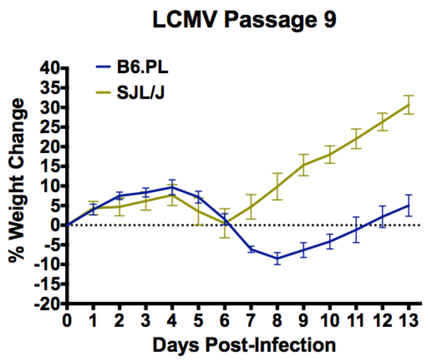


Example of a cell culture plate used to calculate amount of virus per gram of tissue.

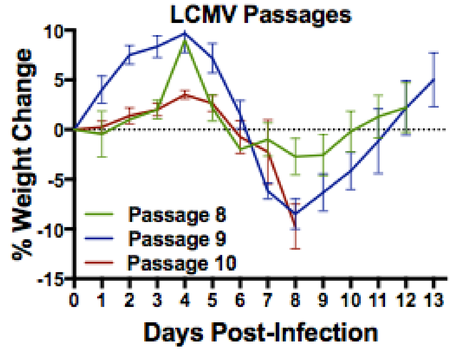
Statistical analysis: The Mantel-Cox log-rank test was used for analysis of Kaplan-Meier survival curves. All statistical evaluations were done using Prism 9 (GraphPad Software, La Jolla, CA).

5. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. American Journal of Hygiene. 1938;27(3):493-7.

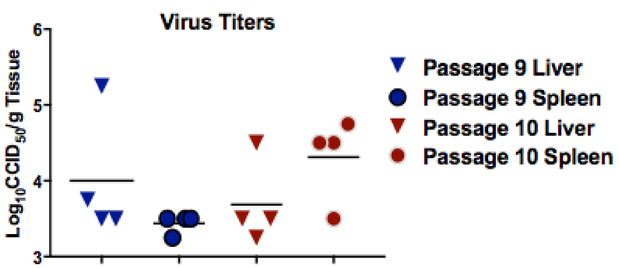
Results



Resulting weights gathered from the 9th passage of the virus, show that the both species and sexes of the infected mice dropped in weight beginning around day 4 post-infection. The SJL/J species began to recover from the virus and gain weight after day 6 post-infection. This species lost about the same weight that they gained during the study, indicating that the virus didn't create much physical ailment.
The B6.PL species also began to lose weight about 4 days post-infection, but they continued to lose weight until day 8. This strain resulted in greater weight loss by body weight percentage, although neither strain resulted in any lethality.



Comparison of the most recent passages report 0% lethality with little weight loss. Passage 9 mice had decreased a considerable amount in weight, but their tissue homogenate wasn't collected until they had been nearly recovered and eliminated most of the virus from their immune system. Passage 10 mice decreased the same percentage of weight, and their tissues were harvested at their lowest weight. Subsequent collection and analyzation of tissue samples of Passage 10 mice presented with a similar quantity of virus.

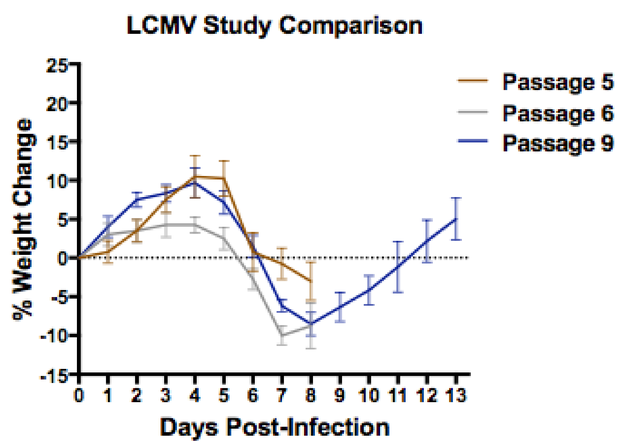


Graphs shown present amount of virus in groups from virus passaging.

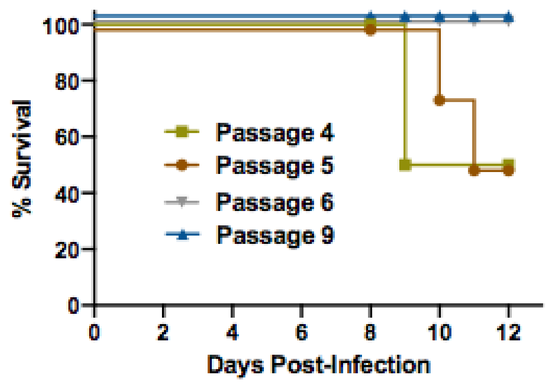
Conclusion

- Lethality of virus decreased with subsequent passages.
- The arenavirus may have mutated to coexist with its natural host to allow viral replication without lethality.
- Continued passaging of the virus will likely not result in a lethal model. Infection of an earlier passage that presented with lethality has a better opportunity of developing 100% lethality.

Study Continuation



This graph compares the recent passaging of Passage 9 without lethality to Passage 5 that presented with lethality and Passage 6 which resulted in tissue collection just prior to death. Comparing these passages aids in observation of discrepancies between studies. While Passage 9 resulted in more dramatic weight change and no death, the earlier passages resulted in almost no weight change with death. Such observation led to the decision to revert to passaging of an earlier stage of collected mutated virus that are capable of lethality.



Further comparison of earlier passages assist in deciding which passage should be selected to continue sequencing the virus. Passages 4 and 5 resulted in clear increased mortality during the experiment. Although not shown by the survival graph, Passage 6 mice were necropsied and tissues collected just prior to death. Passage 6 chosen as the most promising passage to continue mutating the virus.

Management Recommendations for Future Usage of Lethal LCMV Model

- Continue passaging virus until 100% lethality is achieved.
- Harvest organs while mice are at their lowest weight. This is when the virus is at peak replication.
- Tissue homogenate of mice that succumbed from the viral infection will contain the lethal virus strain.
- Lethality designed for B6.PL mouse strain.
- Production of this model will allow more accurate testing of drugs designed for LCMV treatment.

Acknowledgements

We are grateful to Maria Salvato for providing the Clone 13 virus. We thank Kie Hoon Jung for preparing the virus stock for use.