Determining Permissiveness of RVFV MP-12 in Porcine Macrophage Lineage Cells

by

Victoria Cox

Microbiology B.S.

A FINAL HONORS PROJECT

submitted in accordance with the University Honors Program requirements

Biology Department
College of Arts and Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

May 2017

Approved by:

Dr. D. Scott McVey

Abstract

Rift Valley fever virus (RVFV) has a significant impact on ruminants in Africa, leading to high mortality in young animals, frequent abortions in pregnant females, and disease in humans. Although ruminants are the animals chiefly affected by RVFV, it has been shown to infect other animals that could have an impact on its enzootic cycle. Pigs are a common livestock animal that has not been deeply investigated although it has been shown that pig species can possess antibodies to RVFV after inoculation. The objective of this study was to determine if two different porcine derived cell lines of macrophage lineage were permissive to MP-12 virus, an attenuated form of RVFV, due to the lack of information about porcine involvement in infection cycles. Infections at variable multiplicity of infections (MOIs) and viral kinetics showed these cells to be permissive. There was a marked difference in the amount of cytopathic effect (CPE) between the two cell lines with the cells that are more like monocytes showing more CPE than the more macrophage like cells. Both cell lines produced titers ranging from 10^3 to 10^6 plaque forming units/mL (pfu/mL) with comparable titers between the two cell lines. This work serves as a beginning point for deeper investigations into the ability of porcine species to be infected by this disease.

Introduction

Rift Valley fever virus (RVFV: *Bunyavirus, Phlebovirus*) is a virus that is typically found in ruminants and spread via mosquito, but can be passed to humans as well through direct contact with infectious animal fluids and tissues. RVFV has also been shown to cause disease in mice, cats, dogs, camels, and some non-human primates.[1] Symptoms present in the ruminant hosts are abortion storms in herds, nasal discharge, diarrhea and lack of appetite along with the potential for hemorrhagic symptoms.[2] The main targets of infection in cattle and sheep are the liver and spleen along with other secondary lymphoid organs.[3] When looking at the thymus in mice, there was a presence of infected dendritic cells and macrophages present.[4] Although pigs do not have viremia at low doses, it has been shown that they can exhibit viremia at a higher dose and that antibodies can be found in the serum.[5,6] In addition, it was found that porcine kidney and brain cells were permissive to the virus using both wild type and attenuated (MP-12) forms of RVFV.[7] When looking at RVFV, little is known about how pig populations may influence the epizootic cycle. The objective here is to look at whether macrophage lineage cells are permissive to RVFV MP-12 as a potential indication that pigs could be affected by the disease.

Methods and Materials

Cell Culture

The CΔ2+ and CΔ2- cell lines were obtained from Dr. Carol Chitko-Mckown at the U.S. Meat Animal Research Center, Clay Center, Nebraska. The CΔ2- cells were cultured in RPMI media supplemented with 10% FBS, antibiotic/antimycotic, and L-glutamine. The CΔ2+ cells were cultured in RPMI media

supplemented with 10% FBS, 10% supernatant from LM-292 cells, antibiotic/antimycotic, and L-glutamine. Both cell lines were maintained at 37˚C and 5% CO_2.

MOI Infection

24-well plates were seeded at a density of $1x10^5$ cells/well and allowed to incubate for two days to reach near confluency. The wells were infected at the multiplicity of infection (MOI) of 0, 0.01, 0.05, 0.1, 0.5, and 1 with the RVFV MP-12 strain. Supernatants were collected at 24 and 48 hours post infection, mixed 1:1 with BLP, and stored at -80˚C.

Growth Curve

T-25 flasks were seeded at a density of $6x10^5$ cells/flask and incubated for 2 days. Cells were infected at an MOI of 0.1 with RVFV MP-12 strain. Virus was adsorbed for 1 hour and rocked every 15 minutes. After adsorption, virus was removed, the flasks were washed with PBS twice, and fresh media was added. Time course samples were collected at 0, 6, 12, 24, 28, and 72 hours post infection. Supernatant was collected, mixed 1:1 with BLP and stored at -80˚C. Remaining media was removed and the flask was washed with PBS once. Trizol was added directly to the flask, rocked, and the remaining cells scraped to into the Trizol and stored at -80˚C.

Titration

All titrations were done in Vero cells plated 24 well plates using the standard plaque assay method, as follows. The Vero cells were grown in 199E media supplemented with 10% FBS, antibiotic/antimycotic, and L-glutamine. Vero cells were incubated at 37°C until they were confluent (~$0.2*10^6$ cells/well). 1:10 serial dilutions were prepared to move down the plate in 199E media. 100uL of inoculum was placed on each well. The plates were incubated for 1 hour and were rocked every 15 minutes. After incubation 1ml of methyl cellulose was added. The plates were incubated at 37°C for 6 days. After 6 days, the plates were stained with crystal violet and allowed to sit one hour. The plates were washed with DI water and the plaques were counted to calculate titers in plaque forming units per ml (pfu/mL).

Figures

Fig. 1 Comparison of CPE (cytopathic effect) in porcine macrophage lineage cell lines after infection with MP-12 at variable MOIs. CΔ2- and CΔ2+ were infected with MP-12 at MOIs of 0, 0.01, 0.05, 0.1,

0.5, and 1 pictured across the panel. Pictures were taken of the CΔ2- and CΔ2+ at 24 hpi (A and B) and 48 hpi (C and D).

Fig. 2 Comparison of viral titers in porcine macrophage lineage cells after MP-12 infection at variable MOIs. CΔ2- and CΔ2+ cells were infected with MP-12 at MOIs of 0, 0.01, 0.05, 0.1, 0.5, and 1. Titers were calculated via plaque assay of supernatant at 24 hpi (left) and 48 hpi (right). The number of replicates is indicated on each bar.

Fig. 3 MP-12 replication kinetics in porcine macrophage lineage cells. CΔ2- and CΔ2+ cell lines were infected with MP-12 at 0.1 MOI. Titers were calculated via duplicate plaque assays of supernatant at the indicated time point. The mean of two biological replicates is represented.

Results and Conclusions

Two different porcine macrophage lineage cell lines were compared for their permissiveness to the attenuate strain of RVFV, MP-12. Although the main targets of RVFV are the liver, spleen and neurological tissues, it has been shown to replicate in other tissues as well, including lymphoid tissues.[1] When considering the porcine macrophage lineage cell lines, the CΔ2+ cells are more similar to monocytes while the CΔ2- cells are more similar to macrophages. Infection of these cells with MP-12 at different MOIs revealed that the CΔ2+ cells are affected by higher levels of CPE (Fig. 1 B) than the CΔ2- cells (Fig. 1 A) at 24 hours post infection. The same trend can be seen at 48 hours post infection (Fig. 1 D and C). Based on the titers, both cells lines produce a dose dependent response to infection, although the CΔ2+ cells have a decreased titer after 48 hpi at an MOI of 0.1, 0.5, and 1 which is likely due to the high levels of CPE and decreased viral replication (Fig. 2). Using an MOI of 0.1, viral titers peaked at 24 hpi for the CΔ2+ cells and continued to increase through 72 hpi in the CΔ2- cells (Fig. 3). The titers for both cell lines ranged from 10^3 to 10^6 pfu/mL and are comparable between cell lines even though there is a clear difference in the amount of CPE. This would demonstrate that the cell lines are permissible to MP-12 and that these closely related cell lines have different reactions to infections. Given that the CΔ2- and CΔ2+ cell lines from pigs are permissive to MP-12 and the information that pig species have been found to have antibodies to RVFV, more investigation needs to be done to discover the potential impact that pig species have on the RVFV cycle.

References

1. Ikegami, T., & Makino, S. (2011). The Pathogenesis of Rift Valley Fever. *Viruses,3*(5), 493-519. doi:10.3390/v3050493
2. Bird, B. H., Ksiazek, T. G., Nichol, S. T., & MacLachlan, N. J. (2009). Rift Valley fever virus. *JAVMA,234*(7), 883-893.

3. Flick, R., & Bouloy, M. (2005). Rift Valley Fever Virus. *Current Molecular Medicine,5*(8), 827-834. doi:10.2174/156652405774962263
4. Smith, D. R., Steele, K. E., Shamblin, J., Honko, A., Johnson, J., Reed, C., Hensley, L. E. (2010). The pathogenesis of Rift Valley fever virus in the mouse model. *Virology,407*(2), 256-267. doi:10.1016/j.virol.2010.08.016
5. Scott, G. R. (1963). Pigs and Rift Valley Fever. *Nature,200*(4909), 919-920. doi:10.1038/200919a0
6. Evans, A., Gakuya, F., Paweska, J., Rostal, M., Akoolo, L., Vuren, P. V., . . . Njenga, M. K. (2007). Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife. *Epidemiology and Infection,136*(09). doi:10.1017/s0950268807009806
7. Gaudreault, N. N., Indran, S. V., Bryant, P. K., Richt, J. A., & Wilson, W. C. (2015). Comparison of Rift Valley fever virus replication in North American livestock and wildlife cell lines. *Frontiers in Microbiology,6*. doi:10.3389/fmicb.2015.00664