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Morphology and Fitness Differences in Reverse Mutated Strains of the Attenuated rCan Junín Virus in Vero Cell Culture

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Abstract

Argentine hemorrhagic virus Junín (JUNV) is an arenavirus that the WHO has to be a serious public health risk that calls for more attention[5]. There's a live attenuated vaccine available but it isn't approved outside of Argentina due to the instability of the attenuation. The goal for this experiment is to understand how the reverse mutations of the attenuated rCan strain Junín virus affect its fitness and morphology in Vero cell culture. We expect that all four strains of rCan should have similar yield growth, but that they likely will show different plaque morphologies. This work will contribute to the field of JUNV vaccine research that will be represented in a scientific article describing an effort toward developing a safe JUNV vaccine. To test this, we first grew up the virus strains in Vero cell culture and collected samples at different time points. Following collection, we performed a virus titer, and we are currently working on performing a focus forming unit assay of each sample to be evaluated in a growth curve and study the plaque morphology.

Background

Isolate	GP1 168	GP1 186	GP1 206	GP2 427	GP2 446
XJ13	T	E	S	F	T
XJ17	A	E	S	F	T
XJ34	A	E	S	F	S
XJ39, 48	A	E	S	F	S
Candid#1	A	G	P	I	S

rCan #1	Vaccine
rCan #2	I147F
rCan #3	A168T
rCan #4	I147F + A168T

Figure 1. Passage history and development of Candid#1. Changes from XJ13 isolate to Candid #1 are seen in amino acid 168 of glycoprotein 1 and more importantly in amino acid 427 of glycoprotein 2. Taken from Albariño et al.

Junín virus, JUNV, shows similar symptoms to the FLU virus but can have hemorrhagic and neurological problems that appear later during infection which gives JUNV a high mortality rate. An attenuated or weakened version of the virus was made into a vaccine after the virus was isolated from a patient and passed through rodents[1]. The weakening of the virus is caused by small changes in the outside of the virus, specifically the changes of amino acids in the glycoproteins. However, those changes can be unstable. We obtained three reengineered rCan strains from the University of Montana, one with a reverse mutation at amino acid 168 of glycoprotein 1, another with a reverse mutation at amino acid 427 of glycoprotein 2, and the third strain with both mutations.

Methods

Vero Cells are cultured in minimal essential media (MEM) with 5% total volume of Fetal Bovine Serum (FBS), added non-essential amino acids, and added sodium pyruvate. Cells were seeded to its specified confluency in 96 wells and grown overnight in the incubator at 37°C and 5% CO2.
The virus strains were grown up in Vero cell culture and samples were collected at different time points, (Day 0 through Day 6 post infection).
Collected samples were titered in 10-fold serial dilutions and plated again in 96 well plates. The plates were then later read after 7 days to evaluate the presence of virus, following which the cell culture infections dose at 50% death was calculated using Muench's method[3].
On going focus forming unit assays are also being performed with different time point samples grown in microcrystalline methylcellulose, fixed 48 hours post infection, and later detected using antibodies and a NovaRed kit.

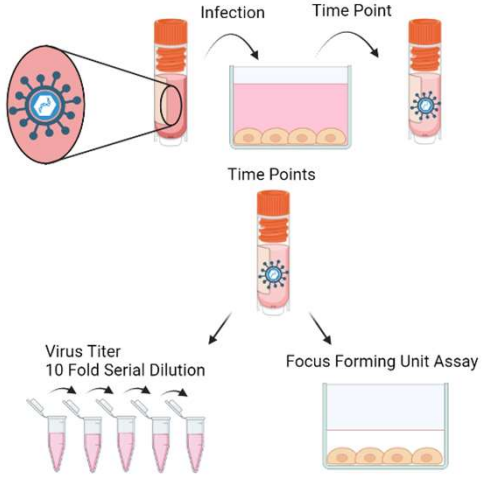

Figure 2. Overview of experiment's methods. First Vero cells were infected with rCan#1-4 and samples were collected for Virus Titers and plaque FFU assays.

Results/Discussion

Following sample collections and a virus titer we found that all four rCan strains do have similar measured yield growth in CCID50 shown in Figure 3. We were also able to grow and visualize plaques from samples of all four strains taken on time point Day 6 using an FFU assay.
We still don't have enough data from the FFU assays to conclude anything on the plaque growth and morphology differences/similarities. Moving forward we are still performing FFU assays from each time point to measure that number of formed plaques and further study the morphology of each rCan strain.

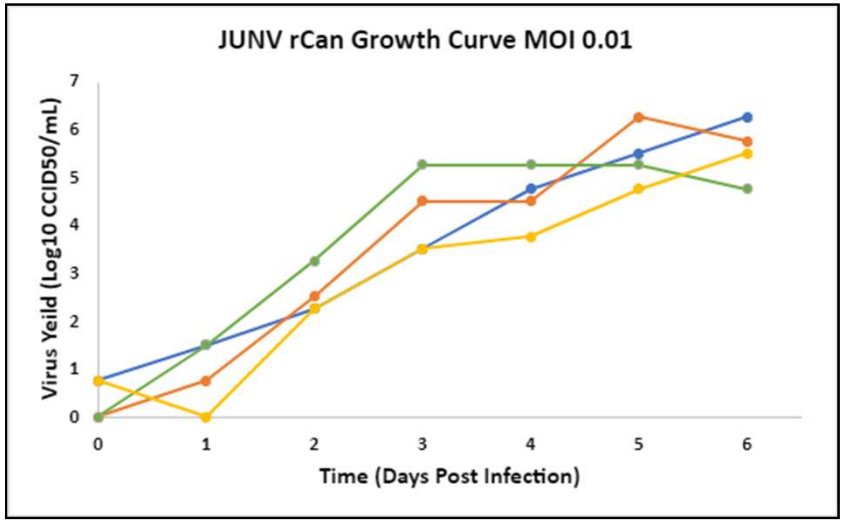

Figure 3. Resulting Cell Culture Infectious Dose at 50% calculated using Muench's method for each time point from virus titer.

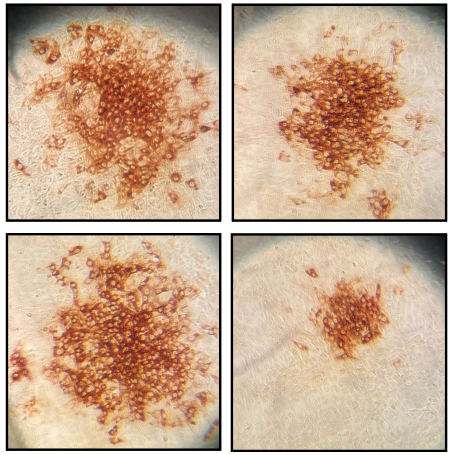

Figure 4. Focus Forming Unit (FFU) assay of rCan #1-4 on Day 6.

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Acknowledgments

1. Albariño, C. G., B. H. Bird, A. K. Chakrabarti, K. A. Dodd, M. Flint, E. Bergeron, D. M. White, andS. T. Nichol. 2011.
2. Gowen, B. B., Hickerson, B. T., York, J., Westover, J. B., Sefing, E. J., Bailey, K. W., ... & Nunberg, J. H. (2021).
3. Muench, H. R. (1938). A simple method of estimating 50 per cent end points. Am J Hyg, 27, 493-497.
4. University of Montana and PI Jack Nunberg
5. WHO 2018, posting date. Annual review of diseases prioritized under the Research and Development Blueprint.