Reverse Mutated Strains of the Attenuated rCan Junin Virus: Differences of Growth Curve Fitness and Growth Morphology

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ABSTRACT

The purpose of this project was to understand how various mutations on the attenuated rCan strain (vaccine strain) of the Junin virus (JUNV) affected its growth fitness and morphology within in-vitro testing. This has previously not been clearly investigated as the JUNV is not prevalent in many societies but due to easy mutation has become a risk. The differences in fitness were determined by comparing the different growth time and pattern in a growth curve assay and the differences in growth morphology was compared using a focus forming unit assay (FFU). There are expected differences in both the growth fitness and morphology between the different strains of the attenuated rCan strains. There is very little known about these various strains of JUNV and how they affect the cells.

INTRODUCTION

The Junin virus (JUNV) is a part of the arenavirus family. It is primarily found in Argentina but has also been found in Africa and the Americas. The arenavirus family has similar symptoms to the FLU virus but can lead to hemorrhagic and neurological problems later in the infection phase. There is an available live-attenuated Candid #1 Junin vaccine (rCan #1), but it is not yet approved in the U.S. The attenuated virus (and the derivatives used in this experiment) come from a series of mutations that causes the changes of single amino acids at various positions within the gene. Due to this easy mutation ability of the Junin virus to the original deadly strain (reverse mutation), JUNV has been considered a serious risk.

OBJECTIVES

Determine the differences between four different attenuated rCan strains of JUNV can affect the growth fitness as well as growth morphology.

This will be compared using:

1. Growth curve assay
2. Focus forming unit assay

METHODOLOGY

Cell Culture and Virus Strains

4 virus strains were prepared and sent to USU IAR by the University of Montana. The four were as follows: rCan #1 (Original), rCan #2 (I147F), rCan #3 (A168T) and rCan #4 (I147F+A168T).

Vero cells were cultured in 5% FBS MEM with added NEAA and NaPyr and grown in a 96 well plate to be grown overnight at 37C and 5% CO.

Growth Curve Assay

This was done by infecting a 96 well plate containing Vero cells with 0.01 MOI of each virus strain. Each day, 1-10, the complete well would be collected and frozen at -80C. After collection was complete, a titration was completed on Vero cells and CPE was done day 7-10. After which the CCID50/mL values were calculated using the Reed-Muench method and put into graphs (Fig. 1).

Focus Forming Unit Assay

One plate containing Vero cells was infected with each strain of rCan at various dilutions and incubated for 2 hours. After which 0.9% MCC overlay was placed on the cells and incubated for 48 hours. The cells were washed and fixed using MeOH/Acetone mixture which was shortly incubated at -20C and then left to dry. The following day, cells were rehydrated and blocked with primary antibodies and incubated for 1 hour. After which, they were washed and more block solution with secondary antibodies was added and incubated for 1 hour. The plates were washed and stained using NovaRED and the FFUs were visualized under a microscope (Fig. 2)

RESULTS

Growth Curve Assay

The growth curve was completed, and CCID50/mL scores were calculated and graphed in Fig. 1. The growth time and fitness seem to be unaffected between the four different attenuated rCan strains of JUNV.

Focus Forming Unit Assay (FFU)

The first round of FFU assays was successful and led to various FFUs as seen in Fig. 2. There seems to be little difference in the morphology between each of the different rCan strains tested. Subsequent FFU assays failed, and no data could be collected.

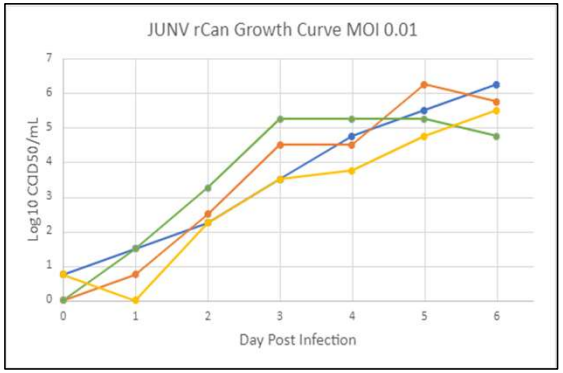

Fig 1. Growth curve of Log10 CCID50/mL. Including rCan #1 (blue), rCan #2 (red), rCan #3 (green) and rCan #4 (yellow).

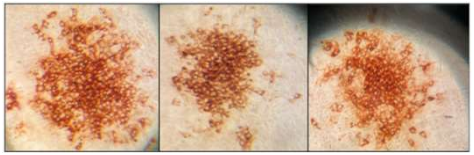

Fig 2. Examples of various focus forming units (unknown rCan strain)

CONCLUSION

Mutations always have an affect on whatever it may be within. In the case of the different mutations in the rCan strains of JUNV, there seems to not be much of a difference in what was seen in these experiments. The growth fitness didn't seem to be affected much between each of the four strains. They follow the same pattern in growth and expression each day. The differences in morphology in undefined yet since the results have yet to be replicated again. Further experimentation and completion of new FFU assays will need to be completed to clearly determine if any differences can be seen between the various rCan strains of JUNV.

Further experimentation in both of these experiments would be beneficial to determine the viral properties of the easily reverse mutated rCan strains of JUNV and therefore being able to assess the potential threat of this virus.

CREDITS

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