Innate Immunity Driven by Incoming Viral Polymerase

Undergraduate Research Opportunities Program

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Introduction

Influenza A viruses (IAV) are negative-sense, segmented RNA viruses that are capable of infecting a number of different hosts, with the potential to cause devastating pandemics. In order to combat these disease epidemics and develop useful vaccines, the mechanism of IAV infection must be comprehensively understood. IAV is made up of eight RNA segments that are released into the host cell as ribonucleoproteins (RNPs). These vRNP complexes traffic to the nucleus and are responsible for both transcription and replication of the viral genome. It is during these early stages of infection that the host innate immune response is triggered through recognition of viral nucleic acids. The goal of this project is to understand the capacity of the incoming vRNP complexes to initiate a host immune response during the early stages of the virus life cycle. This will be done by generating single cycle IAVs (scIAVs) that lack the coding sequence for one of the RNP proteins. Without the ability to make new vRNP complexes, all viral RNA will be generated by the incoming vRNP complexes.

Methods

- **Generation of lentiviral vector**
  - Cloning of lentivirus packaging plasmid using In-Fusion® cloning technique
    - To generate lenti-GFP, lenti-NP, and lenti-IAV-PB1
  - Transfection of HEK293T cells & harvest of lentivirus
    - Transfection of lentivirus packaging plasmid + helper plasmids
- **Transduction of MDCK cells to generate scIAV packaging cell line**
  - Self-inactivating lentivirus will integrate gene of interest + puromycin resistance gene into MDCK genome
  - Lenti-GFP transduction as a control
  - Lenti-IAV-NP & lenti-IAV-PB1
- **Confirmation of integration into MDCK genome**
  - Puromycin selection
  - Genomic DNA
  - Western blot for protein expression

Figure 1.

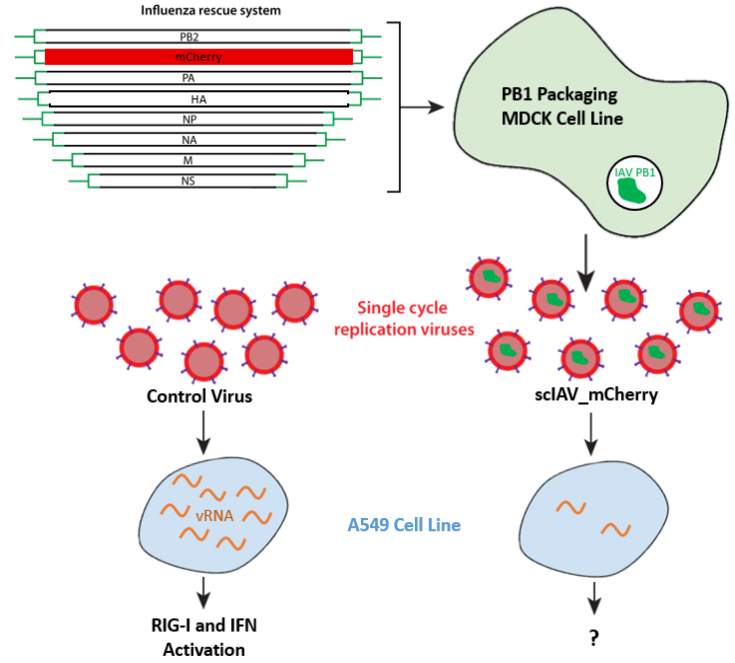

Figure 1. Model system using scIAV_mCherry rescued from PB1_MDCK cell line to look for innate immune activation post infection.

Results

Figure 2

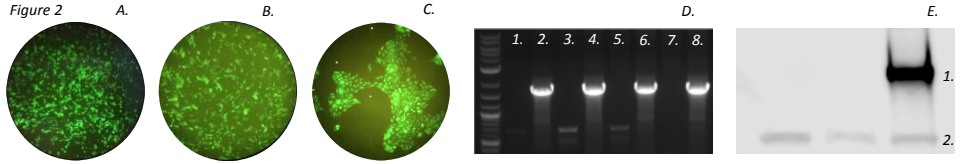

Figure 2: A. Positive control, HEK293T cells post transfection with lenti-GFP. **B.** Positive control, MDCK cells post transduction with lenti-GFP. **C.** Transduced MDCK cells cultured in media containing puromycin. **D.** gDNA extracted from PB1_MDCK cells (wells 1-6) and control MDCK cell line (5-6). PCR amplification of PB1 sequence (even wells) positive control (odd wells). **E.** Western blot of PB1 (1) in PB1_MDCK cell line, actin positive control (2).

Discussion

- GFP expression confirms lentivirus infection in HEK293T cells (*Figure 2A*). Functional lentivirus harvested from cells post transfection.
- GFP expression indicates the lentivirus integration into the cells genome (*Figure 2B*).
- GFP expression shows that all cells capable of growing in the presence of puromycin have undergone lentiviral integration (*Figure 2C*).
- DNA bands in lanes 1, 3, and 5 indicate presence of the sequence of PB1 in packaging cell lines genomic DNA (*Figure 2D*)
- Band indicates the presence of PB1 protein in the PB1_MDCK cell line (*Figure 2E*)

Future Directions

- Perform a two step infusion reaction to generate scIAV plasmids
- Confirm expression of NP in NP_MDCK by gDNA extraction/PCR amplification and western blot
- Rescue of scIAV using 8 plasmid transfection-based system and packaging cell lines
- Use scIAV to analyze the immune response *in vitro*
  - Infection in A549 cells and MLE15 cells
  - Detect activation of RIG-I, a protein involved in detecting vRNA and triggering the host's innate immune response
  - Measure levels of type I interferon (IFN)

References and Acknowledgements

1. Hutchinson, E. C., & Fodor, E. (2013). Transport of the influenza virus genome from nucleus to nucleus. *Viruses*, *5*, 2424–2446. https://doi.org/10.3390/v5102424

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