Creating Diverse Strains of HIV-1 to Investigate Antibody-Dependent Cellular Cytotoxicity in Mother-to-Child Transmission

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Introduction

HIV-1 is an ongoing epidemic, infecting millions of people yearly. With so many different strains of the virus, it is very difficult to create vaccine or find a cure for HIV-1. Over 50% of the world’s HIV infections occur in Sub-Saharan Africa and most of the people in these places do not have access to antiretrovirals. We suspect the antibody-dependent cellular cytotoxicity (ADCC) mechanism as a way for the body to naturally fight the virus. As a result, we applied that mechanism as a way for the body to naturally prevent transmission through breastfeeding.

Methods

- We used single genome amplification using maternal cDNA to isolate the HIV-1 Envelope (Env) sequence. Gel electrophoresis verifies the correct length of the Env, and these Envs are sent for sequencing to ensure complete sequences.
- Yeast Gap repair was used to insert the Env sequence into an isogenic HIV-1 backbone. Yeast Plasma Rescues isolates the yeast DNA which is then transformed in E. coli.
- E. coli containing the HIV-1 plasmid is grown and the plasmids are then isolated from the culture.
- Using a spectrophotometer, we measured the plasmid concentration of each sample.
- HIV-1 plasmids were transferred into 293t cells transfected with reagent Fugene-6 or Polyethyleneimine (PEI).
- Virus was titered using a T2M assay to determine the relative light units emitted by the virus.

Results

- In order for virus to be successful, the relative light units (RLU) of the titer needs to buy higher than the negative RLU.

Discussion

- During yeast gap repair, we picked either 1 or 4 yeast colonies to see if there is a higher chance of creating virus from picking single or bulk colonies.
- After failing to make virus in the first attempt, we changed the chemical used in transfection. Instead of using Fugene-6, we used PEI for the following titers.

Conclusion

- When picking either 1 or 4 colonies, both showed results as patient 1666’s virus was made by picking 1 colony and 1732’s virus was made by picking 4 colonies.
- Using PEI in transfection showed better results as more viruses were made using PEI than Fugene-6.
- In the future, 293t virus will be passaged through activated CD4 cells in order to make higher titer virus for use in future ADCC assays.

References


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