Understanding VACV A35R Functions in MHC Class I and II Antigen Presentation

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Background

- Within the poxvirus family, the orthopoxvirus genus is of great importance because of its substantial threat to human populations via zoonotic transmissions (e.g. mpox).
- A35R has been previously described in the vaccinia virus (VACV) to inhibit MHCII presentation, and the deletion virus was attenuated
- *There is a lack of continued study on how A35R modulates both MHC class I and II antigen presentation and the similarities in functions between the different poxviruses.*

Establishing Stable Cell Line

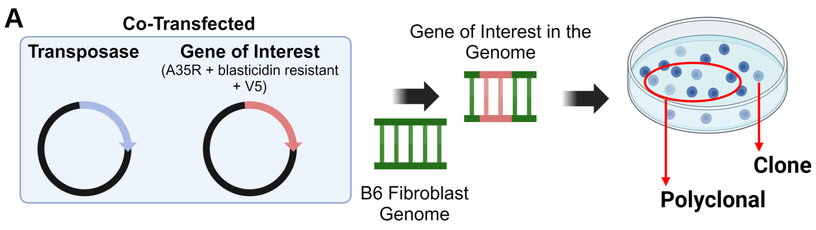
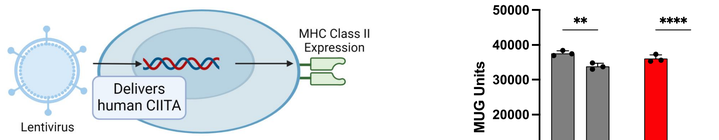


Figure 1: (A) General overview of establishing an inducible (through doxycycline) cell line. **(B)** Western blotting analysis also demonstrates a noticeable increase in molecular weight in ECTV A35R compared to VACV A35R perhaps due to post translational modification. **(C)** We found that a polyclonal fibroblast cell line stably expressed the A35 protein in a graded manner.

A35R and MHC Class II Impact and Interaction



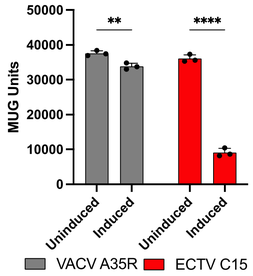


Figure 2: (A) The general method used to induce MHC class II molecules. **(B)** The induction of MHC class II was successful onto the fibroblast cells as the MUG units decreased with A35R induction, matching the literature trend. ECTV C15, another conserved immunoregulatory protein in the poxvirus known to downregulate class II antigen presentation, was placed as a control.

A35R and MHC Class I Impact and Interaction

MUG Hybridoma Assay

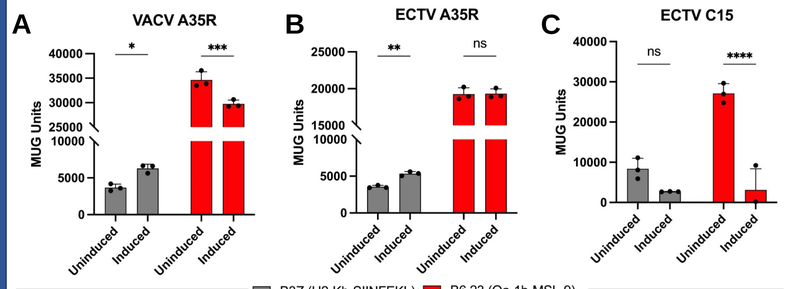


Figure 3: A35R selectively modulates non-classical MHCI antigen presentation in VACV and increases classical MHCI presentation for both ECTV and VACV. T cell hybridoma assays were conducted on (**A**) VACV A35R and (**B**) ECTV A35R. (**C**) ECTV C15-expressing cell line was the control.

Flow Cytometry

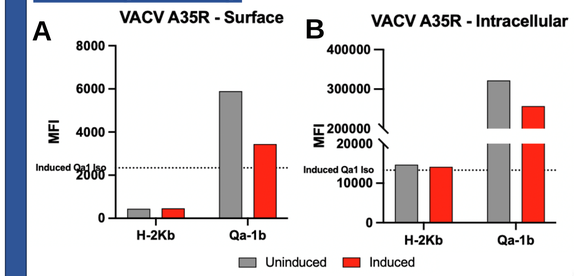
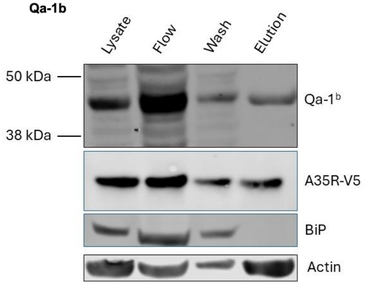


Figure 4: Flow cytometry experiment indicates that nonclassical antigen presentation decreases both on the surface of the cell and intracellularly when cells are induced.

Co-Immunoprecipitation (VACV)

Figure 5: Co-Immunoprecipitation was conducted with the anti-V5 agarose. The Western blot analysis demonstrates a potential direct interaction between Qa1b and A35R. The actin band in the elution lane gives additional possible functions of A35R's role.



Identifying Amino Acids Responsible for PTM

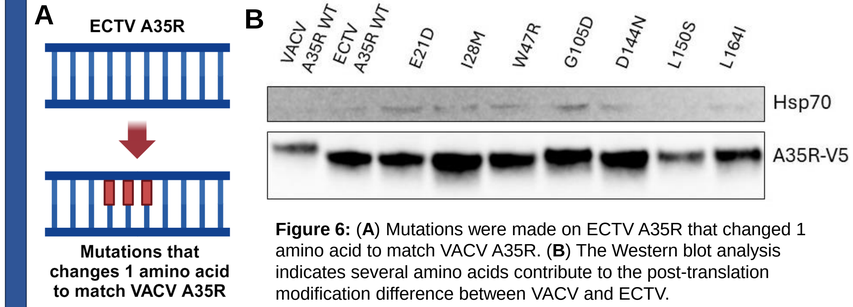


Figure 6: (**A**) Mutations were made on ECTV A35R that changed 1 amino acid to match VACV A35R. (**B**) The Western blot analysis indicates several amino acids contribute to the post-translation modification difference between VACV and ECTV.

Generating VACV and ECTV A35R mRNA

Figure 7: Transfection of the putative mRNA molecules demonstrates the success of creating mRNA for A35R through (**A**) western blotting and (**B**) flow cytometry.

Conclusion

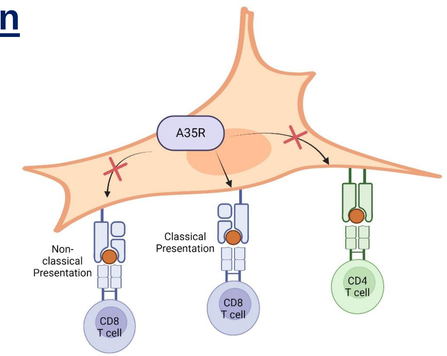


Figure 8: Summary of the interactions that A35R exhibits on MHC Class I and II antigen presentation in VACV: A35R modulates the MHC class I nonclassical and MHC class II presentation unlike MHC class I classical presentation.

Future Directions

- Continue investigating the post-translational modification between ECTV and VACV cell line and the purpose it has on the virulence factor.
- Investigate the differing effects that A35R has on MHC class I classical and nonclassical presentation.
- Continue investigating the effects that A35R has on MHC class II antigen presentation.
- Utilize mRNA to allow for robust expression transiently in diverse cell types (e.g. dendritic cells)
- Generating lentiviral A35R Vectors to test point mutants in MUG assays.

References

- Brennan, G. (2022). *mBio, 14*.
- Forsyth, K. (2020). *PLoS pathogens, 16.*
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Acknowledgments

Members of the Eisenhlor Lab