The Role of IRF5 in Innate Immune Sensing of HIV-1 icRNA

Aidan Joseph[1,2], Sita Ramaswamy[2], Andrés Quiñones[2], Suryaram Gummuluru[2]

Half Hollow Hills High School East, 50 Vanderbilt Pkwy, Dix Hills, NY 11746[1], Boston University School of Medicine, 650 Albany Street, Boston, MA 02118

Introduction

- Combined Antiretroviral Treatment (cART) is effective in people living with HIV (PLWH) in preventing progression to AIDS

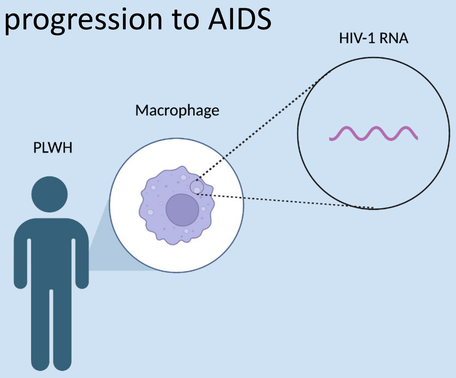

Figure 1: HIV-1 RNA has been found in macrophages isolated from PLWH & Residual Immune activation persists despite cART resulting in HIV-associated non-AIDS (HANA) clinical conditions

- Innate immune sensing of HIV-1 intron-containing RNA (icRNA) leads to pro-inflammatory responses in macrophages yet pathway unknown
- Knockout study of transcription factor Interferon Regulatory Factor 5 (IRF5) shows it has a role in HIV-1 infected macrophages
- Goal: Aim to characterize the role of IRF5 in the HIV-1 icRNA innate immune sensing pathway through gain of function experiments

Methods

Transduction of Jurkat Cells

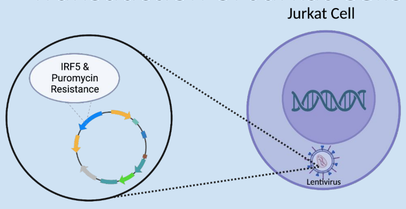

Figure 2: Visual diagram of transduction of Jurkats.

Western Blot Analysis to confirm expression of IRF5 & other proteins involved in innate immune sensing

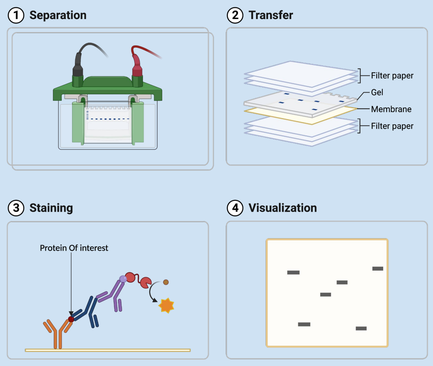

Figure 3: Visual diagram of western blot.

ELISA to measure cytokine production

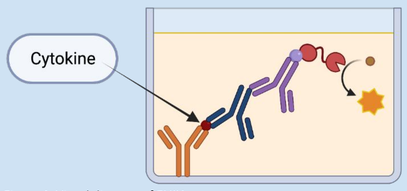

Figure 4: Visual diagram of ELISA.

RT- qPCR to measure cytokine production

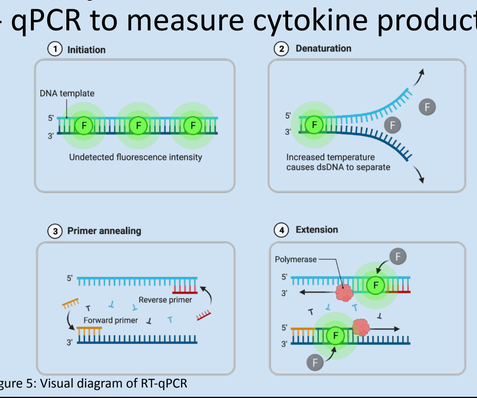

Figure 5: Visual diagram of RT-qPCR

Results

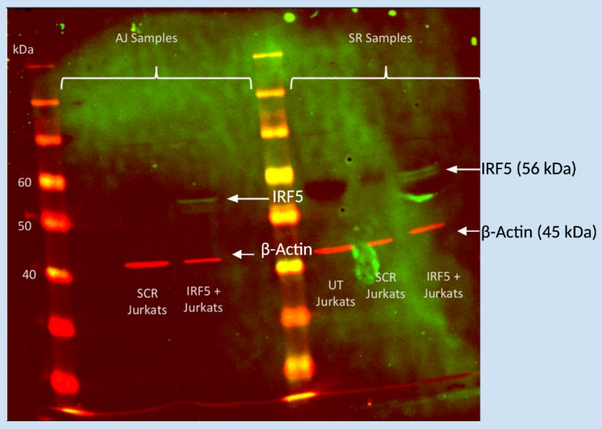

Figure 6: Western Blot of Parental, Scrambled, and IRF5 transduced cells. Demonstrates that transduction of Jurkats was successful.

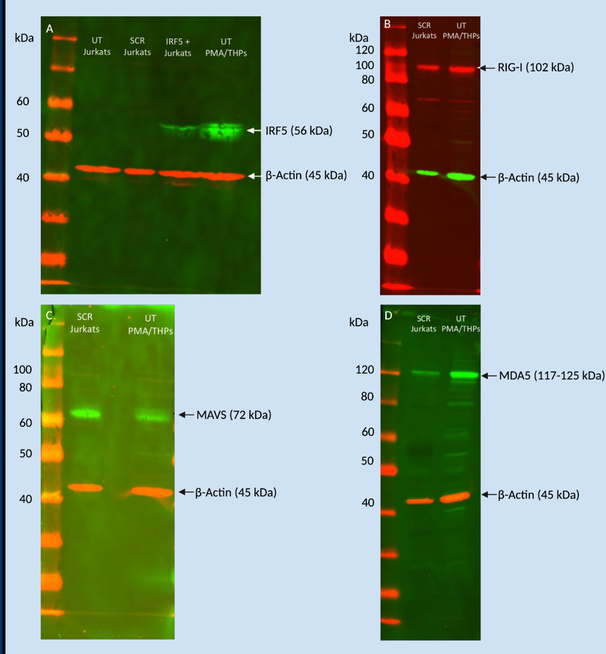

Figure 7: Western blots with β-Actin as a control. **a** Western blot for IRF5 in parental, scrambled, IRF5 transduced Jurkats, and PMA/THP-1 cells. PMA/THP-1 cells acted as a positive control for IRF5. Shows successful transduction of Jurkats as shown by IRF5 band. **b**,**c**,**d** Western blot of Rig-I, MAVS, and MDA5 to see if proteins involved in innate immune sensing are present in Jurkats.

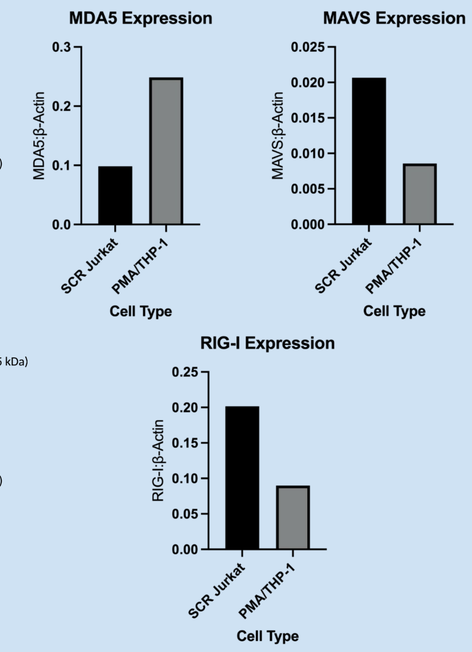

Figure 8: Expression levels of MDA5, MAVS, and RIG-I normalized to β-actin.

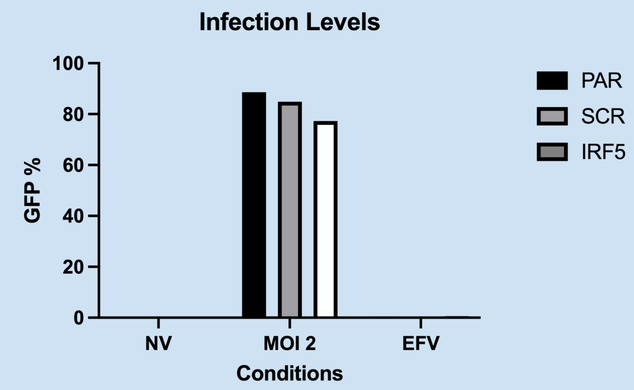

Figure 9: Infection levels of the samples. GFP was a reporter for infection levels.

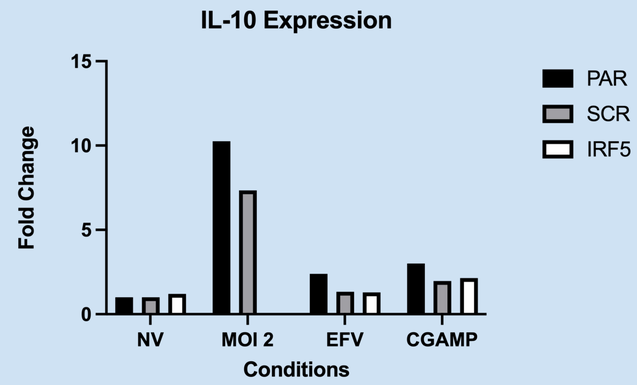

Figure 10: Fold change in anti-inflammatory cytokine IL-10 expression. Conditions NV (no virus), MOI 2 (Multiplicity of infection 2), EFV (Efavirenz), and CGAMP (Cyclic GMP-AMP) were tested. IL-10 expression increases in response to infection. IRF5 virus sample had low quality RNA resulting in no IL-10 being detected.

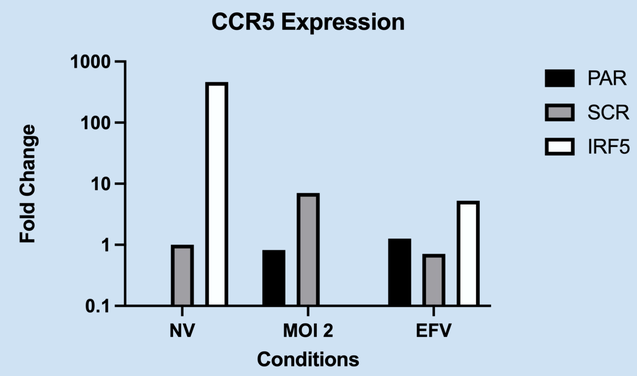

Figure 11: Fold change in co-receptor CCR5 expression. No values for NV PAR condition so everything normalized to NV SCR. CCR5 Expression increases in IRF5 transduced cells without virus treatment but does not when treated with EFV. IRF5 virus sample had low quality RNA similar to figure 6 so no CCR5 was detected.

Discussion/ Conclusions

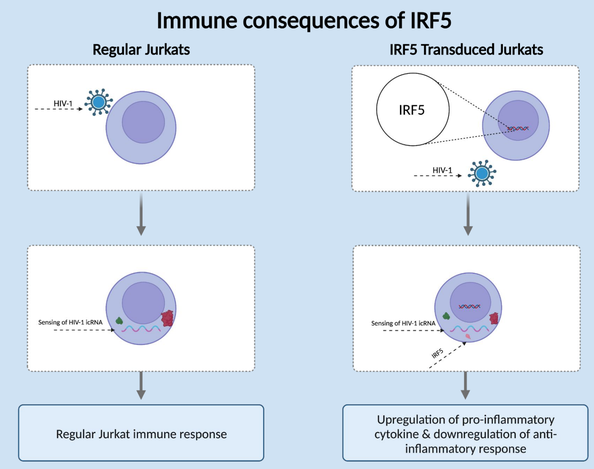

Figure 12: Proposed mechanism of IRF5's role in innate sensing of HIV-1 icRNA.

- Jurkats were successfully transduced to express IRF5 as shown by immunoblots
- RIG-I, MAVS, & MDA5 shown to be expressed in Jurkats
- RT-qPCR of IL-10 & CCR5
  - IRF5 may have some effect on CCR5 expression (EFV inhibits increase in expression)
- Work aids in better understanding of immune consequences of chronic HIV-1 infection
- Future Directions Repeat Infections, use other ligands such as Imiquimod as a TLR 7 agonist as positive controls, and look at other cytokines such as IFN-γ & CXCR4

References

Akiyama, H.; Miller, C. M.; Ettinger, C. R.; Belkina, A. C.; Snyder-Cappione, J. E.; Gummuluru, S. HIV-1 Intron-Containing RNA Expression Induces Innate Immune Activation and T Cell Dysfunction. Nature Communications 2018, 9 (1). https://doi.org/10.1038/s41467-018-05899-7.

Akiyama, H.; Jalloh, S.; Park, S.; Lei, M.; Mostoslavsky, G.; Suryaram Gummuluru. Expression of HIV-1 Intron-Containing RNA in Microglia Induces Inflammatory Responses. 2021, 95 (5). https://doi.org/10.1128/jvi.01386-20.

Acknowledgements

I would like to thank Sita, Andrés, and Dr. Gummuluru for their mentorship and guidance throughout my time here. Additionally, I would like to thank Dr. Sagar & Dr. Gummuluru for hosting journal club which granted me a new perspective on the field of science. I would also like to thank the BU rise program for providing this opportunity.