The On-Going Conflict Between APOBEC3 Immune Factors and HIV-1 Vif

UNIVERSITY OF SASKATCHEWAN
College of Medicine
DEPARTMENT OF BIOCHEMISTRY, MICROBIOLOGY AND IMMUNOLOGY
MEDICINE.USASK.CA

Tanvir Minhas, Amit Gaba, Linda Chelico

University of Saskatchewan, College of Medicine

Department of Biochemistry, Microbiology, and Immunology

INTRODUCTION

- **APOBEC3 (A3) enzymes** are a family of **cytosine deaminases**; they act to remove an amine group from cytosine, deaminating it to a uracil.[1] Of the 7 human A3s, 4 are linked to inhibition of HIV viral spread: A3D, **A3F**, **A3G**, and A3H.[2]
- A3 enzymes can act on ssDNA (-) that was reverse transcribed from HIV's genomic ssRNA (+) to induce G→A mutations.
- There are two ways in which A3s can inhibit HIV viral spread via the induced mutations:

  1. Triggering the base excision repair mechanism of the target cell to degrade the highly deaminated proviral DNA before it is integrated into the host genome.

  2. Inhibiting the formation of virions needed for gRNA transport by producing defective viral proteins from deaminated proviral DNA.

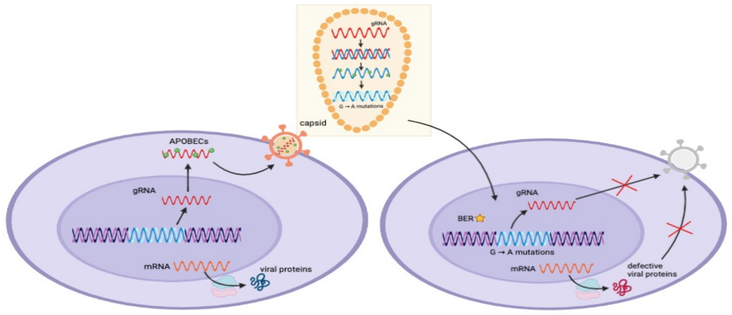

Figure 1. Diagram displaying viral spread of HIV and its inhibition by A3s.

- **Virion infectivity factor (Vif)** is the viral antagonist of APOBEC3. Vif hijacks the Cullin 5 E3 ubiquitin ligase complex to target APOBEC3s, tagging them for degradation via the proteosome pathway.

Gaba et al., Viruses, 2021

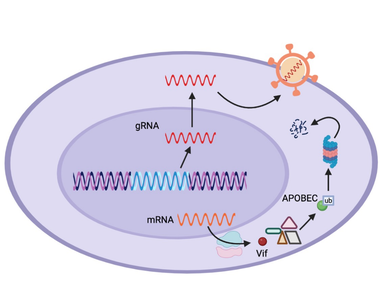

Figure 2. The antagonistic actions of Vif against APOBEC3s.

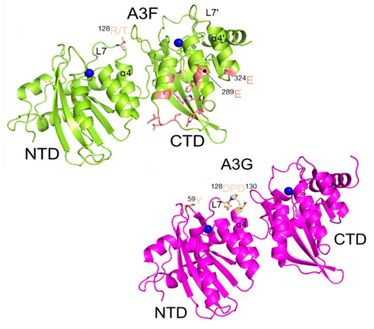

Figure 3. Protein structures of A3F and A3G.

- Recent studies have noted the formation of **Transmitted/Founder (TF) viruses**: viral strains of HIV that form within 6 weeks of transmission and have phenotypes unique from those of the donor's chronic strain.[3]
  - TF viruses form through bottlenecks present during transmission.
- Past investigations using **lab adapted isolated (LAIs)** of HIV showed that interaction between A3G and A3F provides a protective factor, making A3F less susceptible to Vif-mediated degradation.[4]

Mohammadzadeh et al., Heliyon, 2019

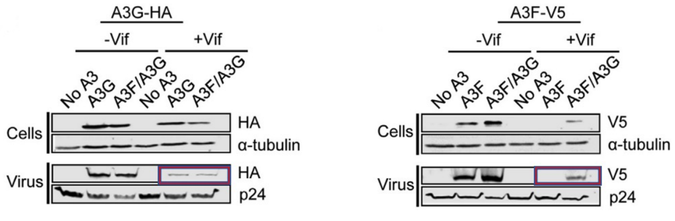

Figure 4. Western blot results displaying A3G and A3F protein concentrations in various conditions to inform the influence of A3 co-expression on Vif-mediated degradation. [4]

HYPOTHESIS

Co-expression of A3F and A3G makes A3F less susceptible to degradation by Vif from clinically isolated Transmitted/ Founder viruses.

METHODOLOGY

1. Cloning: integration of the TF Vif sequence into pcDNA plasmid.
2. Transfection: of 293T cells with Vif- and A3-containing plasmids.
3. Harvest: collection of transfected cell lysates.
4. Immunoblotting: qualitative measurement of protein abundance using cell lysates.

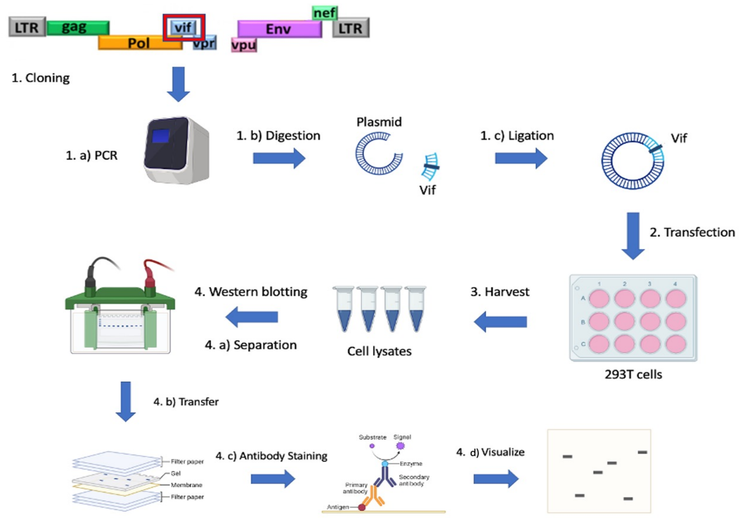

Figure 5. Schematic representation of the study's methodology.

RESULTS

- Positive cloning results were obtained for eight of the eleven TFs.
- Transfection of 293T cells and subsequent cell lysate harvesting and immunoblotting of TF40 showed the protective effects of A3F and A3G interaction.

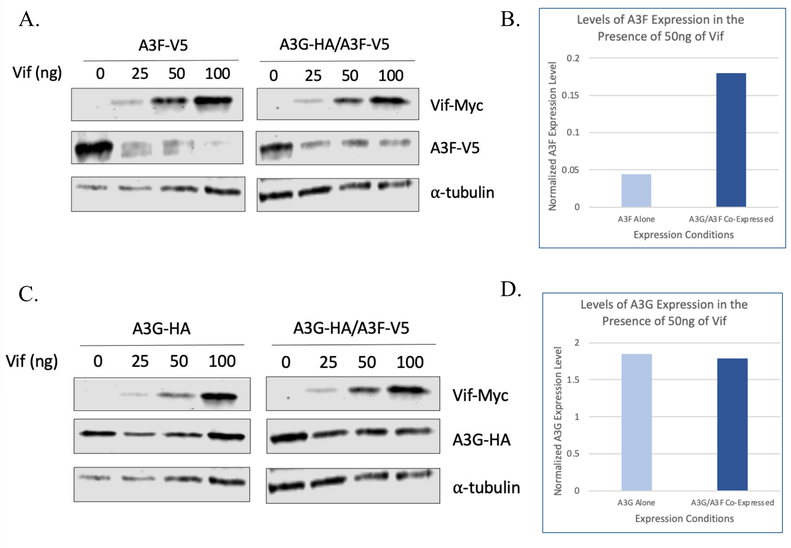

Figure 6. Protein levels for A3F and A3G when they are either expressed alone or co-expressed, and when in the presence of varying TF40 Vif amounts. Results are displayed qualitatively with western blots (A., C.) and quantitatively with graphs (B., D).

CONCLUSIONS

- Western blot results from TF40 support the hypothesis.
- The four-fold increase in A3F protein abundance when expressed with A3G showcases the protective effect of A3F/A3G co-expression on A3F Vif-mediated degradation.

FUTURE DIRECTIONS

- Future directions: continuing the cloning protocol to obtain plasmids for all clinical isolates of interest, then following the subsequent steps in our methodology to characterize APOBEC3-Vif interactions.
- Assuming similar results that uphold our hypothesis in the future, there are implications for the development of Vif inhibitors which can serve as HIV treatment.
  - Advantageous because such treatments could help avoid the plethora of side effects associated with current treatments such as antiretroviral therapy.

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CONTACT

Inquiries can be directed to tanvir.minhas@usask.ca