Chobanian & Avedisian School of Medicine
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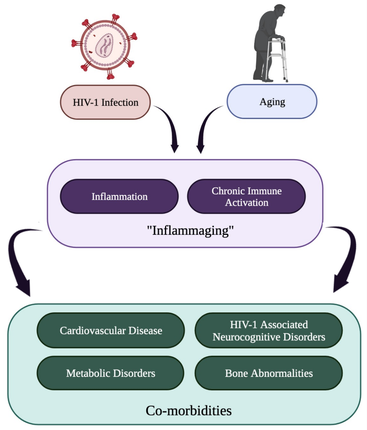
IRF5 Mediates Persistent Inflammatory Responses in HIV-1 Infected Macrophages

[1]Sita Ramaswamy, [1]Jacob Berrigan, [1] Hisashi Akiyama, [1]Andrés Quiñones, [1]Archana Asundi, [1]Alex Olson, [1]Yunhan Chen, [1]Yan Mei Liang, [1]Manish Sagar, and [1]Rahm Gummuluru
[1]Department of Virology, Immunology, and Microbiology , Boston University School of Medicine, Boston, MA, USA

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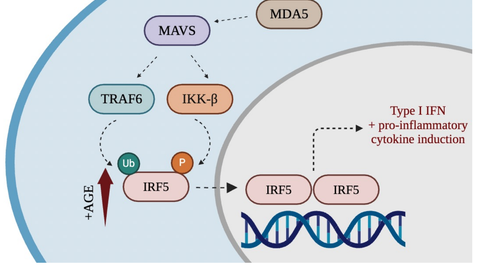
Introduction

- Although anti-retroviral therapy (ART) has significantly extended the lifespan of people with HIV, chronic immune activation persists in these individuals
- HIV RNA and DNA has been shown to persist in long lived patient macrophages (Kandathalil, 2018, *J Clinical Invest)*
- Previous data indicates that HIV-1 intron containing RNA (icRNA) leads to innate immune activation in macrophages (Akiyama, 2018, *Nat Comm)*
- Interferon Regulatory Factor 5 (IRF5) is a major regulator of pro-inflammatory cytokine expression in response to a variety of stimuli, including viruses such as HSV-1 and influenza (Almuttaqi, 2019, *FEBS)*

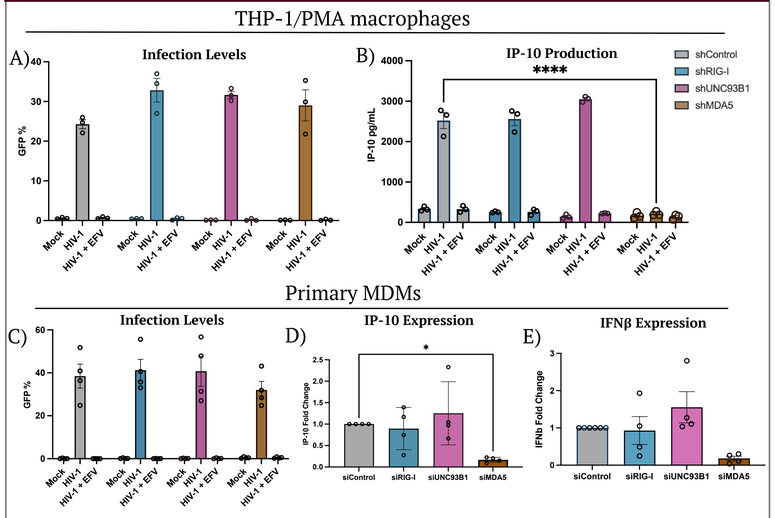


Central Hypothesis

We propose that persistent IRF5 activation downstream of the viral icRNA sensing pathway in infected macrophages contributes to the "inflammaging" phenotype observed in aging people with HIV

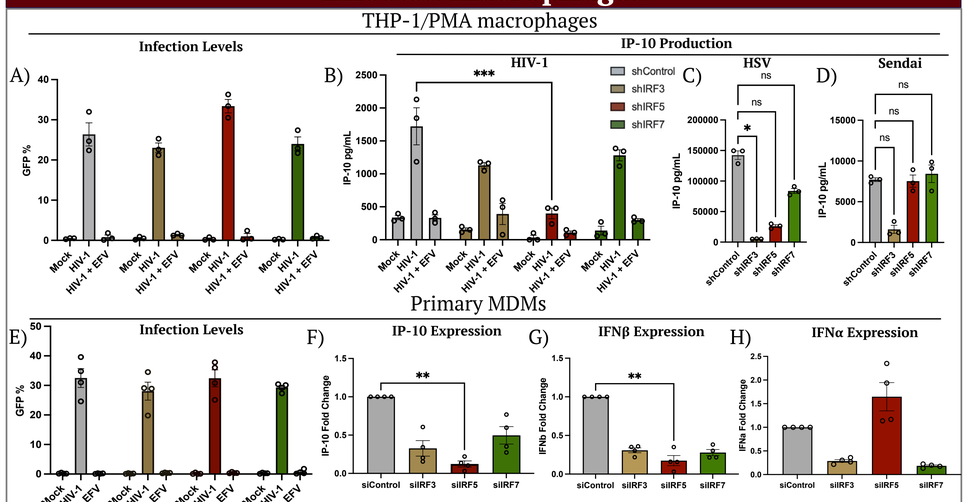


MDA5 is required for HIV-1 induced IP-10/ IFN production in macrophages



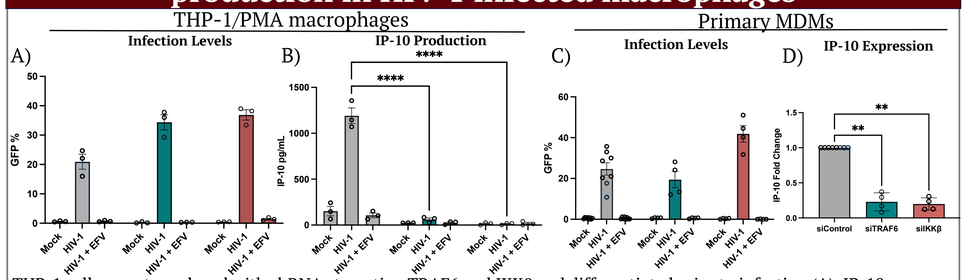
Monocytic THP-1 cells were transduced with an shRNA lentiviral vector to knock down RIG-I, UNC93B1, or MDA5 expression. THP-1 cells were differentiated using PMA and infected with reporter HIV-1 in the presence or absence of RT inhibitor (EFV)(A). Response to infection was measured via IP-10 ELISA(B). Primary MDMs were treated with siRNA prior to infection with reporter HIV-1 (C) and IP-10 and IFNb expression was measured 2 dpi. (D-E)

IRF5 is an important mediator of IP-10 expression in HIV-1 infected macrophages



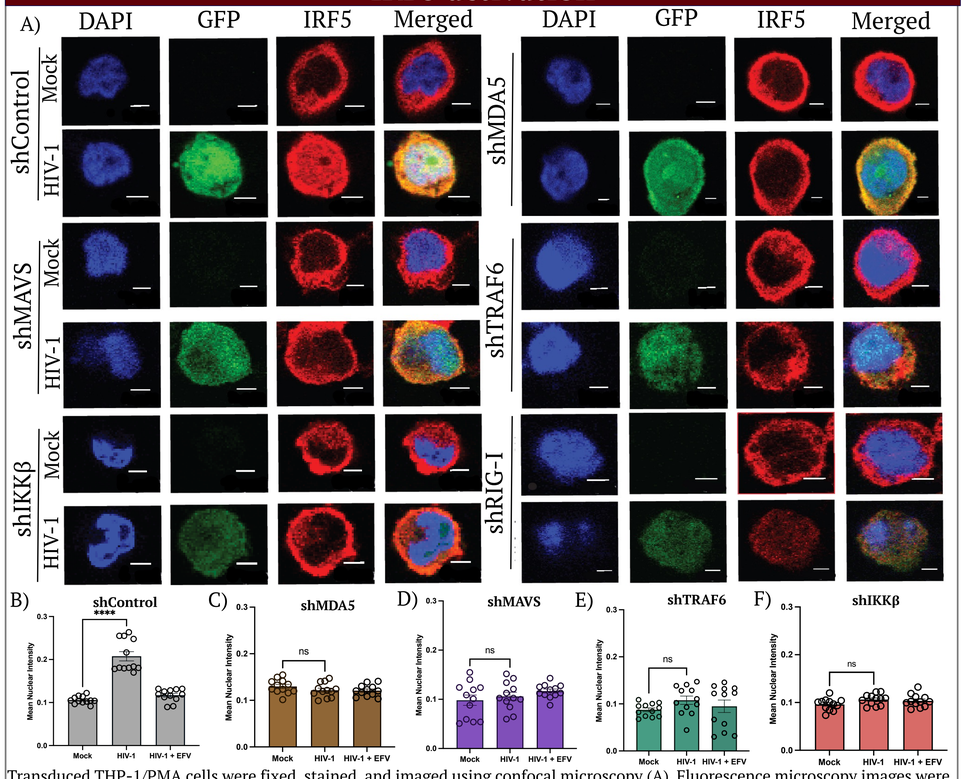
THP-1 cells were transduced with shRNAs targeting IRF3/5/7 and differentiated prior to infection in the presence or absence of RT inhibitory (EFV) (A). IP-10 was measured 3 dpi (B) and cells were also infected with either HSV or Sendai with IP-10 secretion measured 2 dpi (C-D). siRNA treated MDMs were also infected (E) and IP-10, IFNβ, and IFNα was measured via RT-qPCR (F-H)

TRAF6 and IKKβ are required for HIV-1 induced IP-10 production in HIV-1 infected macrophages



THP-1 cells were transduced with shRNAs targeting TRAF6 and IKKβ and differentiated prior to infection (A). IP-10 was measured 3 dpi (B). siRNA MDMs were infected with reporter HIV-1 (C) and IP-10 expression was measured 2 dpi (D).

MDA5, MAVS, TRAF6 and IKKβ are required for HIV-1 induced IRF5 activation



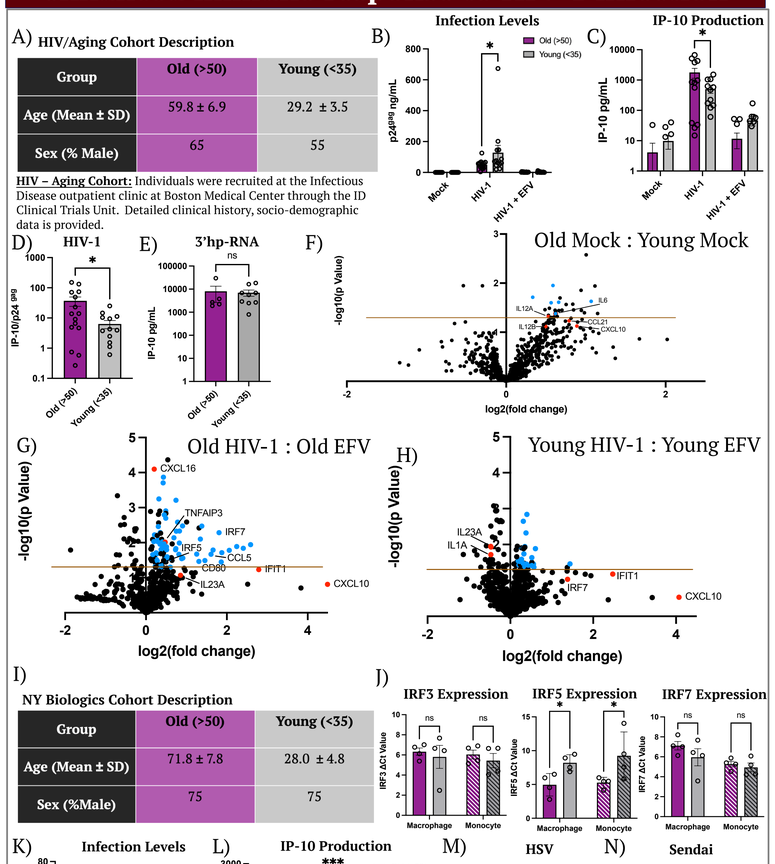
Transduced THP-1/PMA cells were fixed, stained, and imaged using confocal microscopy (A). Fluorescence microscopy images were run through a CellProfiler pipeline in order to quantify IRF5 nuclear localization across knockdown conditions with each dot representing a field containing 40-80 cells(B-F)

Macrophages from older individuals display an enhanced immune response to HIV-1 infection

A) HIV/Aging Cohort Description

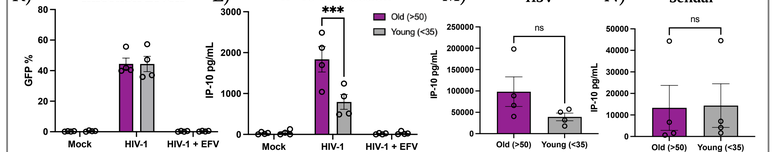
Group	Old (>50)	Young (<35)
Age (Mean ± SD)	59.8 ± 6.9	29.2 ± 3.5
Sex (% Male)	65	55

HIV – Aging Cohort: Individuals were recruited at the Infectious Disease outpatient clinic at Boston Medical Center through the ID Clinical Trials Unit. Detailed clinical history, socio-demographic data is provided.



I) NY Biologics Cohort Description

Group	Old (>50)	Young (<35)
Age (Mean ± SD)	71.8 ± 7.8	28.0 ± 4.8
Sex (%Male)	75	75



MDMs isolated from participants without HIV-1 (A) were infected with reporter HIV-1 and p24[gag] (B), and IP-10 was measured 6 dpi (C-D). As a positive control, cells were treated with 3'hpRNA (RIG-I agonist) for 18 hrs followed by IP-10 quantification (E). RNA lysates from 10 individuals (5 old, 5 young) were analyzed via Nanostring to assess expression of myeloid innate immunity at baseline (F) and upon HIV-1 infection (G-H). MDMs and monocytes from a different cohort of individuals without HIV-1 (I) were assessed for baseline IRF3, IRF5, and IRF7 expression via RT-qPCR (J). IP-10 production was quantified 3 dpi for HIV-1 infection (K-L) and 2 dpi for HSV/Sendai (M-N).

Conclusions

- MDA5 plays a role in the sensing of HIV-1 icRNA in macrophages
- IRF5 is a mediator of IP-10 production in HIV-1 infected macrophages
- TRAF6 and IKKβ play a role in IRF5 activation and HIV-1 induced IP-10 production in macrophages
- Macrophages from older individuals exhibit higher expression of ISGs in response to HIV-1 infection
- Future studies will aim to characterize the role of IRF5 in CD4+ T cells as well as further elucidate age-related differences in macrophage response to HIV-1 infection

Acknowledgements

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