The cellular pathway and mechanisms of IFNa8 induced HIV reactivation in vitro

Authors:
Mvaya L[1,2], Royle C[1], Hong Y, Luo M[1,2], Zhao Y[1], Graham M[3], Roche M[4], Lewin S[4,5], Wang L[6], Davidson KC[6], Duette G[1,2], Saksena MM[1,2], Pelligrini M[6,7], Cunningham AL[1,2], Nasr N[1,2]*

1. The Westmead Institute for Medical Research, Centre for Virus Research, Westmead, Australia
2. The University of Sydney, Sydney Infectious Diseases Institute, School of Medical Sciences, Faculty of Medicine and Health, Sydney, Australia
3. Synapse Proteomics, Children's Medical Research Institute, University of Sydney, Westmead, Australia; School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Sydney, Australia.
4. Department of Infectious Diseases, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia.
5. Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Australia.
6. Division of Infectious Disease and Immune Defence, The Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia; Department of Medical Biology, The University of Melbourne, Melbourne, VIC, Australia.
7. Centenary Institute Medical Research Foundation, Camperdown, Sydney, NSW 2050, Australia

Background: HIV is transcriptionally silent in long-lived resting CD4 T cells that are invisible to immune clearance under lifelong antiretroviral therapy (ART). This latent reservoir is the barrier to HIV cure because it drives rapid viral rebound when ART is interrupted. Reactivating latent HIV is therefore a major strategy for a cure. We previously identified IFNα8 as an inducer of HIV reactivation in primary CD4 T cells invitro, but the mechanism underlying this effect is not yet defined.

Aim: To define the IFNα8 signaling pathway and proteins mediating HIV reactivation.

Methods: Memory CD4 T cells were infected with HIV_{BaL} and then were either treated with IFNα8 alone or with proteins inhibitors to 4 signaling pathways: JAK–STAT, CRKL–STAT5, PI3K/mTOR, or PI3K/NF-κB. HIV replication/reactivation was quantified as p24+ cells by flow cytometry. Mass spectrometry defined the proteomic and phosphor-proteomic response to IFNα8-induced reactivation, while confocal microscopy assessed nuclear localization of potential STAT proteins involved in HIV reactivation.

Results: Pharmacological inhibition specifically Fludarabine, a STAT1 inhibitor, identified JAK–STAT signaling as the pathway for HIV reactivation. Mass spectrometry

confirmed the involvement of this pathway via the activation/phosphorylation of STAT1 in addition to STAT3-6 following IFNα8 treatment. It also revealed distinct IFNα8-driven proteomic profiles: downregulated proteins contributing to HIV reactivation while upregulated proteins are involved in limiting HIV spread and preventing apoptosis of infected cells. Confocal microscopy revealed nuclear translocation of phosphorylated (p) STAT1, STAT4 and STAT6 but not pSTAT3 and pSTAT5. Finally, we showed that IFNα8 did not reactivate HIV ex vivo in CD4 T cells from people living with HIV (PLH) and in a humanized mouse model of HIV latency.

Conclusion: FNα8 induces HIV reactivation in vitro but fails to reactivate the HIV reservoir in CD4 T cells derived from PLH on ART or a mouse model of HIV latency.

Disclosure of Interest Statement: N/A