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Session: **BS2**: Friday May 1 – 15:00:17:00 – HIV Latency and Viral Reservoirs

Track:	Basic Sciences
Subject:	HIV Latency and Viral Reservoirs
Presentation Type:	Oral
Title of Abstract:	**HIV-1 Nef enhances viral reactivation from latency by stimulating TNF-α expression**
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Abstract

Background: A better understanding of host and viral factors that influence mechanisms of HIV latency and reactivation will be critical to improve efforts to eradicate infection. HIV Nef is an abundant early viral protein that modulates T cell signaling events, but its role during viral reactivation from latency remains unclear.

Methods: We established a latent HIV-infected human CEM T cell clone harbouring a single integrated provirus encoding functional Nef (C-Lat-Nef) and a corresponding clone in which *nef* was knocked out using CRISPR/Cas9 (C-Lat-Nef-KO). Viral reactivation (Gag-p24) and tumour necrosis factor alpha (TNF-α) expression was measured by flow cytometry following stimulation of cell with a latency reversing agent (LRA) (prostratin or panobinistat).

Results: Following treatment with prostratin, we observed higher levels of Gag-p24 expression (% of cells $p24^+$) in C-Lat-Nef compared to C-Lat-Nef-KO cells ($P<0.001$). Similar results were found using panobinostat and other C-Lat clones. Interestingly, we also observed higher levels of intracellular TNF-α following LRA stimulation in C-Lat-Nef compared to C-Lat-Nef-KO ($P<0.0001$). Addition of a TNF-α-blocking antibody to LRA-stimulated cultures of C-Lat-Nef cells markedly decreased Gag-p24 expression. Treatment of C-Lat-Nef cells with TAPI-2, an inhibitor of pro-TNF-α converting enzyme ADAM17, similarly diminished Gag-p24 expression ($P<0.001$). Finally, we observed that addition of exosomes containing Nef was sufficient to trigger TNF-α expression and viral reactivation in C-Lat-Nef-KO cells.

Conclusion: Our results demonstrate that Nef enhances viral reactivation from latency through a mechanism mediated by TNF-α. These observations are consistent with Nef's ability to promote the formation of ADAM17-containing exosomes that may stimulate TNF-α-mediated signaling pathways upon binding to or ingestion by target cells. This work highlights a novel role for Nef in modulating latent HIV infection.