IFNα activates latent HIV in non-proliferating latently infected T-cells

Renée M. van der Sluis1, Nitasha A. Kumar1, Vanessa A. Evans1, Andrew N. Harman2, Talia M. Mota1, Surekha Tennakoon1, Paul J. Herzog3, Sharon R. Lewin4,5 and Paul U. Cameron1,4

1The Peter Doherty Institute for Infection and Immunity, The University of Melbourne and Royal Melbourne Hospital, Melbourne, Australia; 2Centre for Virus Research, Westmead Millennium Institute, Westmead, New South Wales, Australia; 3Centre for Inmate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia; 4Department of Infectious Diseases, Alfred Health and Monash University, Melbourne, Australia.

**Background**

Pegylated IFN-α2b monotherapy in HCV/HIV co-infected individuals off antiretroviral therapy (ART) can lead to a reduction in cell associated (CA) HIV DNA1,2, while in HIV-infected individuals on ART, there is a reduction in CA unspliced HIV RNA3 and CA DNA4. It is unclear if IFNα targets the latent virus directly or if the reduction in viral DNA or RNA is due to IFN-induced immune activation. We asked whether IFNα had an effect on the establishment, maintenance and reversal of HIV latency using an *in vitro* model of HIV latency using blood isolated resting CD4+ T-cells and myeloid (m)DC5,6.

**Aims**

1. Determine the effect of increasing concentrations of IFNα on productive infection
2. Determine the effect of increasing concentrations of IFNα on latency establishment
3. Determine the effect of IFNα on latency reversal

**Methods**

**Cell isolation**

PBMC → Magnetic Bead Depletion → CD8, CD11b, CD14, CD16, CD19, CD56, DR

Resting CD4+ T cells

Dendritic Cells

Flow cytometry cell sorting

Myeloid DC

Proliferation dye eFluor670

**Cell culture set-up**

- eFluor670 labelled CD4+ T-cells + mDC
- eFluor670 labelled productive infection
- eFluor670 labelled Latent infection

**Figure 1.** Resting CD4+ T-cells are negatively selected using magnetic cell sorting, stained with the proliferation dye eFluor670 and cultured alone or with syngenic mDC (DC: T cell ratio of 1:10) ± IFNα and infected with full-length nef competent eGFP-reporter virus. At day 5 post infection productive infection is determined by detecting eGFP+ cells using flow cytometry and the non-productively infected (eGFP-) and proliferating (eFluor670+) CD4+ T-cells were sorted. Sorted T-cells were cultured with the integrase inhibitor, raltegravir (RAL) in the presence or absence of an activation stimulus (anti-CD3/CD28+IL-7+IL-2) ± IFNα. Cells were harvested 72 hrs after stimulation and eGFP expression was measured by flow cytometry. To quantify latency infection the number of eGFP+ cells in the unstimulated culture (background) was subtracted from the number of eGFP+ cells following stimulation.

**Results**

**IFNα reduces productive infection and establishment of latent infection in non-proliferating T-cells**

**Figure 2.** IFNα-induced inhibition of productive and latent HIV infection. Resting CD4+ T-cells co-cultured with mDC were treated with type I IFNα. Productive and latent infection was determined in non-proliferating (A) and proliferating (B) T-cells and the percentage inhibition was calculated (C, D). Columns represent median values and dots represent individual donors (n=11-12 donors), lines indicate mean values ±SEM. Statistics were determined with a paired student T-test (*p<0.05, **p<0.01, ***p<0.001).

**IFNα activates latent HIV in non-proliferating T-cells**

**Figure 3.** IFNα activates latent HIV. Resting CD4+ T-cells were co-cultured with mDC and infected as described above, eGFP non-proliferating (eFluor670+) and proliferating (eFluor670+) T-cells were sorted, cultured in the presence of RAL and either left unstimulated, cultured with 100 U/ml IFNα, treated with anti-CD3/CD28 or activated with anti-CD3/CD28 in the presence of 100 U/ml IFNα for 3 days. eGFP expression was quantified by flow cytometry. Red bars indicate median values and dots represent individual donors (n=3-4 donors), lines indicate mean values ±SEM. Statistics were determined with a paired student T-test (*p<0.05, **p<0.01, ***p<0.001).

**Conclusions**

**Using an *in vitro* latency model we have shown that:**

1. IFNα can inhibit productive infection and the establishment of HIV latency
2. Once latency is established IFNα can reverse latency in non-proliferating T-cells and inhibits the effects of anti-CD3/28 on reversing latency

**Future work**

1. Determine which IFN-stimulated genes and/or virus restriction factors inhibit productive infection and latency establishment. Trim19 and trim 22 are of particular interest as they have been shown to inhibit HIV transcription.
2. Investigate how IFNα can act as an latency reversing agent. We hypothesise that IFNα-induced activation of the JAK/STAT signalling pathway induces HIV transcription.