Plasmacytoid DCs control HIV latency in resting T-cells by type I IFNα

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A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Background

We previously demonstrated that myeloid DC (mDC), but not plasmacytoid DC (pDC), induce HIV latency in T-cells *in vitro*[1,2]. Since pDC produce high levels of type-I interferon (IFN), we asked whether different IFNs have an effect on the establishment, maintenance and reversal of HIV latency in CD4+ T cells.

Aims

1. Determine the effect of increasing concentrations of IFNα on productive infection

2. Determine the effect of increasing concentrations of IFN on latency establishment

3. Determine the effect of IFN on latency reversal

Methods

In vitro cell culture set-up

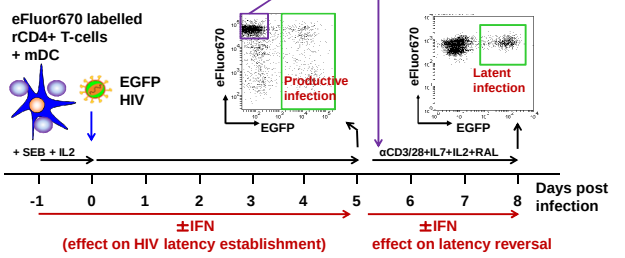

Figure 1. Effect of IFN on HIV latency establishment and reversal in vitro. Resting CD4+ T-cells are negatively selected using magnetic cell sorting, stained with the proliferation dye eFluor670 and cultured alone or with syngeneic mDC (DC: T cell ratio of 1:10) ±IFN and infected with full-length nef 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-), non-proliferating (eFluor670^{hi}) CD4+ T-cells were sorted. Sorted T-cells were cultured with integrase inhibitor raltegravir (RAL) in the presence or absence of activation stimuli (anti-CD3/CD28+IL-7+IL-2) ±IFN. Cells were harvested 72 hrs after stimulation and EGFP expression was measured by flow cytometry. To quantify latent infection the number of EGFP+ cells in the unstimulated culture (background) was subtracted from the number of EGFP+ cells following stimulation.

Ex vivo cell culture set-up

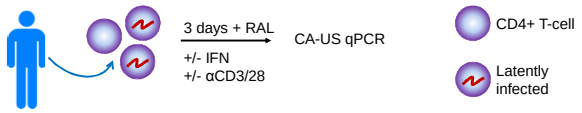

Figure 2. Effect of IFN ex vivo on viral transcripts in CD4+ T-cells from HIV-infected individuals on ART. Total CD4+ T-cells isolated from PBMCs collected by leukapheresis from HIV-infected individuals on ART, were cultured in the presence of integrase inhibitor left untreated or treated with IFN, activated with anti-CD3/CD28 or activated with anti-CD3/CD28 IFN for 3 days. RNA was isolated, reverse transcribed into cDNA and cell-associated (CA) unspliced (US) HIV was measured by qPCR.

Results

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

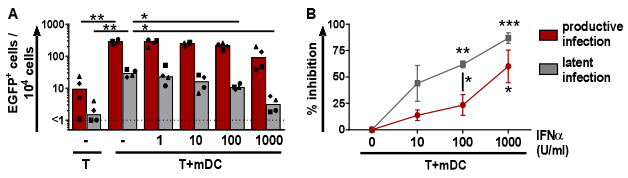

Figure 3. IFNα-induced inhibition of productive and latent HIV infection. Resting CD4+ T-cells co-cultured with mDC were treated with type I IFNα. Productive and latent infection was determined in non-proliferating T-cells (**A**) and the percentage inhibition was calculated (**B**). Columns represent mean values and dots represent individual donors (n=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).

Results

Type I IFNα, IFNβ and IFNω inhibit establishment of latent infection

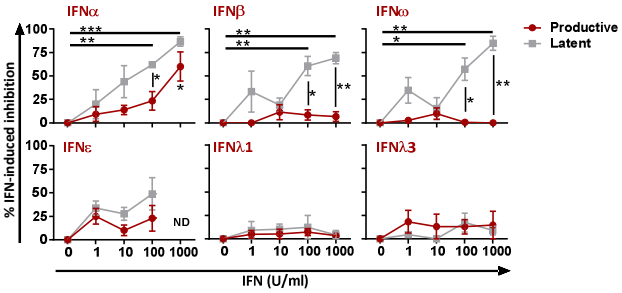

Figure 4. IFN-induced inhibition of productive and establishment of latent infection. Resting CD4+ T-cells were co-cultured with mDC and indicated concentrations of type I IFNα, IFNβ, IFNε, IFNω or type III IFNλ1 or IFNλ3. **A:** Productive (dark grey) and latent (light grey) infection in non-proliferating T-cells was determined as described in figure 3 and the IFN-induced inhibition was quantified. Lines indicate mean values ±SEM of 3-4 donors. *p<0.05, **p<0.01, ***p<0.001, as determined by paired student T-test.

IFNα activates latent HIV *in vitro*

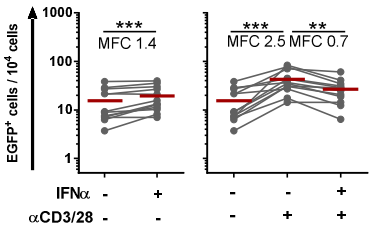

Figure 5. IFNα activates latent HIV. Resting CD4+ T cells were co-cultured with mDC, infected and EGFP- non-proliferating T-cells were sorted. Sorted cells were cultured in the presence of RAL and either left untreated, cultured with 100 U/ml IFNα, activated with anti-CD3/CD28 or activated with anti-CD3/CD28 in the presence of 100 U/ml IFNα for 3 days. EGFP expression was quantified by flow cytometry. Red bars indicate mean values and dots represent individual donors (n=12). Statistics are determined with paired student T-test (**p<0.01, ***p<0.001). MFC = mean fold change.

IFNα activates latent HIV *ex vivo*

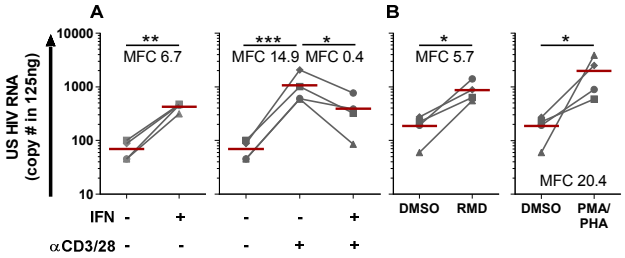

Figure 6. IFNα induces expression of unspliced HIV RNA *ex vivo*. Total CD4+ T-cells isolated from HIV-infected individuals were cultured in the presence of integrase inhibitor and left untreated or treated with 100 U/ml IFNalpha, activated with anti-CD3/CD28 or activated with anti-CD3/CD28 + 100 U/ml IFNalpha (**A**), or treated with DMSO, romidepsin (RMD) or PMA+PHA (**B**) for 3 days and unspliced (US) HIV was measured by qPCR. Red bars indicate mean values and dots represent individual donors (n=4). *p<0.05, **p<0.01, ***p<0.001, as determined by paired student T test on log-transformed data. MFC = mean fold change.

Conclusions

1. Using an *in vitro* latency model we have shown that IFNα can inhibit productive infection and can inhibit the establishment of HIV latency. Similar results were obtained with IFNβ and IFNω.

2. Once latency *is established* IFNα, but not IFNβ or IFNω, can reverse latency in T-cells and inhibits the effects of anti-CD3/28 on reversing latency *in vitro* and *ex vivo*

Implications

IFNα effects on control of latency are complex. It can act as an latency reversing agent but can also block productive infection in activated cells and the virus production induced by TcR stimulation. Understanding these mechanisms may allow targetted use of IFN.

References

1. Evans *et al.* Myeloid Dendritic Cells Induce HIV-1 Latency in Non-procR stimulatioliferating CD4+ T Cells. Plos Pathogens 2013 **10**(10):e1004486.
2. Kumar *er al.* The role of antigen presenting cells in the induction of HIV-1 latency in resting CD4+ T-cells. Retrovirology 2015 **12**:76