Targeting type I interferon mediated immune activation to control chronic HIV infection *in vivo*

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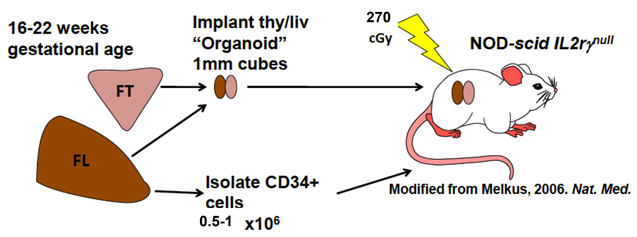
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ABSTRACT

Chronic immune activation, immunosuppression and T cell exhaustion are hallmarks of HIV infection, yet the mechanisms driving these processes are unclear. As chronic activation can be a driving force in immune exhaustion and type I interferons (IFN-I) are emerging as critical components underlying ongoing activation in HIV infection, we tested the effect of blocking IFN-I signaling during chronic HIV infection. Using HIV infected humanized mice, we demonstrate that blockade of IFN-I signaling during chronic HIV infection in vivo diminished HIV-driven immune activation, decreased T cell exhaustion marker expression, restored HIV-specific CD8 T cell function, and led to decreased viral replication. Antiretroviral therapy (ART) in combination with IFN-I blockade further decreased viral loads and reduced the latent HIV reservoir compared to ART treatment alone. Our data suggest that blocking expression of IFN-I in conjunction with ART treatment can restore immune function and may reduce viral reservoirs during chronic HIV infection.

Bone Marrow /Liver/Thymus (BLT) mice



- Reconstitution comprised of multiple lineages
- Sustained, high level T cell development
- T cells educated on autologous thymic tissues
- Detectable T and B cell responses to HIV infection

Expression of exhaustion and activation markers are elevated on T cells in chronically infected BLT mice

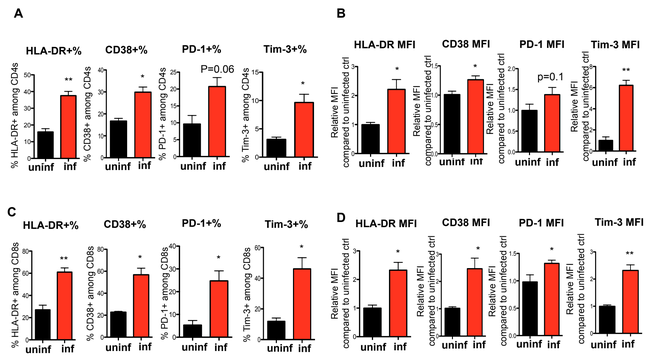


Figure 1. Chronic HIV infection results in elevated expression of activation and exhaustion markers and exhaustion of viral specific CD8 cells. NSG-hu BLT humanized mice were constructed by implanting fetal liver and fetal thymus as well as hematopoietic stem cells (HSCs) into the NSG mice. After human immune reconstitution, mice were mock infected or infected with HIVNL4-3. 13 weeks after infection, whole blood from each mouse were collected and cells were stained with anti-human antibodies CD45, CD3, CD4, CD8, Tim-3, PD-1, and HLA-DR and analyzed by flow cytometry. Expression of HLA-DR, CD38, PD-1, Tim-3 on human CD4 (**A&B**) and CD8 cells (**C&D**) was accessed by gating and by measuring the % positive and relative MFI of marker expression (n=3-5 mice per group). For relative MFI, Data displayed is the relative MFI ± SEM of these markers as compared to the mean MFI of uninfected controls, showing differences between these populations.

Type I IFN signaling is chronically elevated during HIV-1 infection

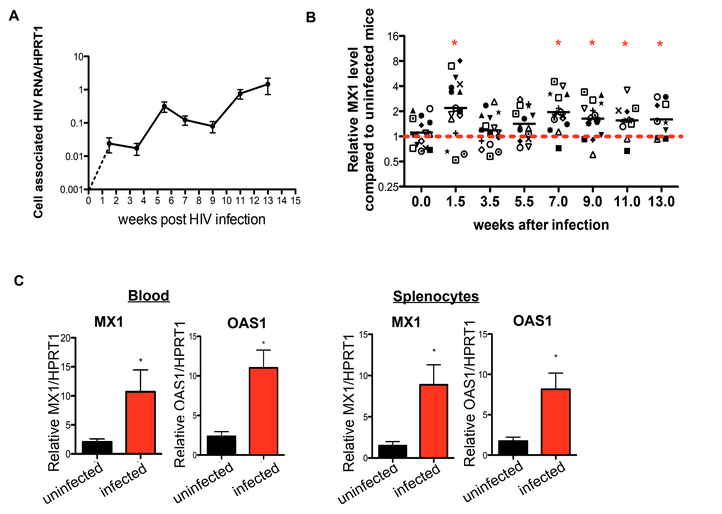


Figure 2. Type I IFN signaling is chronically elevated during HIV-1 infection. A) Change of HIV cell associated RNA levels in PBMCs from BLT mice after HIV infection as compared to human housekeeping gene hypoxanthine phosphoribosyltransferase 1 (HPRT) expression (n=7). **B)** Expression level of MX1 gene in PBMCs throughout chronic HIV-1 infection. Expression level of MX1 and OAS1 in **C)** PBMCs and **D)** splenocytes from uninfected and infected mice as measured by RT-PCR. (* p<0.05)

IFNR blockade effectively lowers ISG expression levels and reverses immune exhaustion

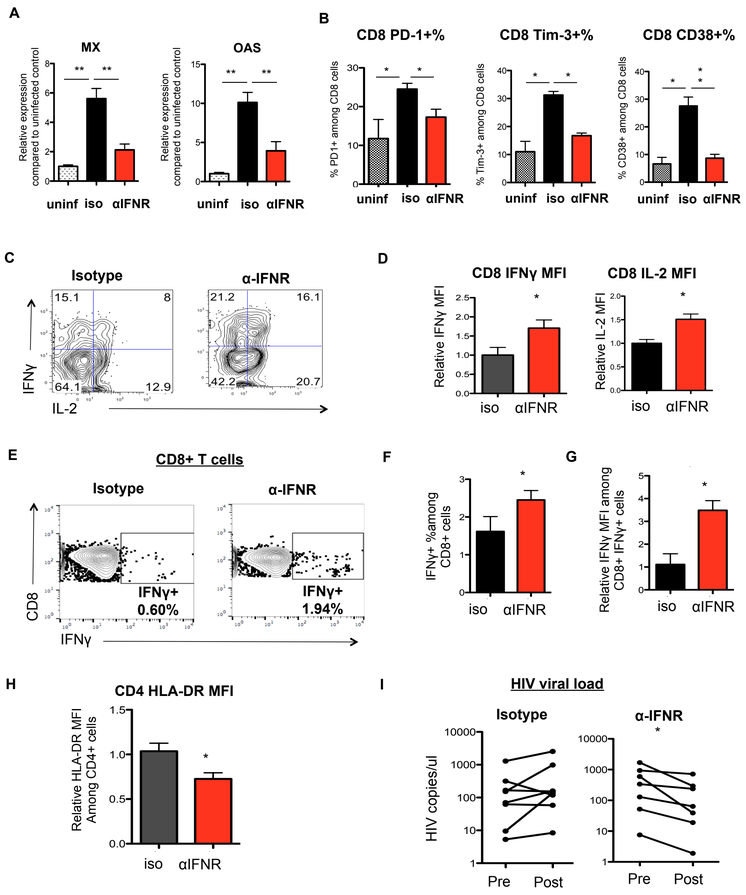


Figure 3. IFNR blockade effectively lowers ISG expression levels and reverses immune exhaustion. A) 12 weeks after infection, mice were treated with IFNR blocking antibody (clone MMHAR-2) (n=7) or an isotype antibody control for 8 days (n=7). Expression levels of ISGs MX1 and OAS1 in the BLT humanized mouse human PBMCs after treatment were measured by real time RT-PCR. **B)** PD-1, Tim-3, and CD38 expression on T cells from HIV infected mice that were treated or mock treated with IFNR blocking antibody or uninfected mice. **C)** Splenocytes from uninfected, HIV-1 infected, HIV-1 infected and IFNR antibody treated mice were stimulated with PMA/ionomycin for 6 hours. Intracellular production of IFNγ and IL-2 by CD8 were measured by intracellular staining and flow cytometry. **D)** Summary of cytokine production data by PMA/ionomycin stimulated CD8 cells described in in Fig 3C as measured by relative MFI. **E)** Splenocytes from HIV-1 infected, HIV-1 infected and IFNR antibody treated mice were stimulated with HIV clade B peptide pool (pol, gag, env and nef) and production of IFNγ by viral specific cells were measured by flow cytometry. **F)** Summary of the total percentage of IFN-γ+ cells among human CD8+ cells after HIV peptide pool stimulation. **G)** Summary of IFN-γ production by human CD8+ cells as measured by relative MFI of expression after HIV peptide pool stimulation. **H)** Summary of relative HLA-DR MFI on CD4 T cells from blood and spleen of infected mice that were treated with isotype or type I IFNR antibody. **I)** Changes of plasma viral load before and after isotype treatment or IFNR blockade. (NS: not significant; * p<0.05)

Concurrent IFNR blockade ART further reduces type I IFN signaling and results in faster viral suppression and reduced viral reservoir

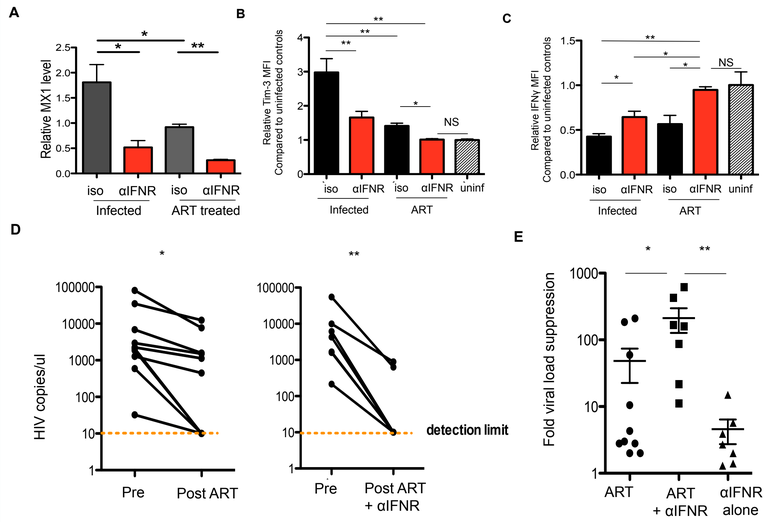


Figure 4. IFNR blockade treatment with ART reduced IFN-I signaling and promoted faster viral suppression. HIV infected mice were either treated with an isotype control antibody (iso) (n=7), treated with IFNR antibody (αIFNR)(n=7), or treated with 2-3 weeks of ART (n=9) or with combined therapy of ART and IFNR antibody (n=6). **A)** MX1 expression levels from infected mice in different treatment group as measured by PCR. **B)** Relative Tim-3 expression level of CD8 T cells in PBMCs from infected mice in different treatment group as compared to uninfected mice. **C)** Relative IFNγ MFI of PMA/ionomycin stimulated CD8 T cells from infected mice in different treatment group as compared to uninfected mice. **D)** Changes of viral load in HIV-1 infected NSG-huBLT mice that were treated with ART or ART plus IFNR blockade. **E)** Fold viral suppression after ART treatment, ART with IFNR blockade or IFNR blockade alone.

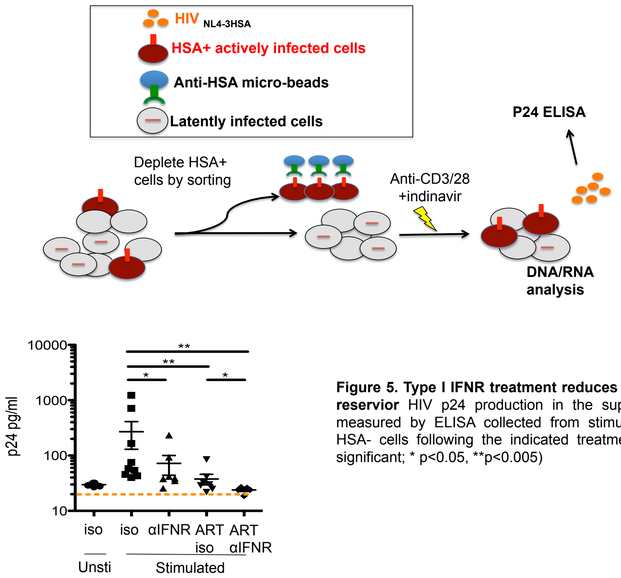


Figure 5. Type I IFNR treatment reduces viral latency reservior HIV p24 production in the supernatant (as measured by ELISA collected from stimulated, sorted HSA- cells following the indicated treatment. (NS: not significant; * p<0.05, **p<0.005)

Conclusions

- Similar to what is observed in humans, HIV infected BLT mice have elevated expression of activation and exhaustion markers
- Type I IFN signaling is chronically elevated during HIV infection.
- Type I IFN receptor blockade, particularly during ART treatment could restore functions of exhausted T cells and facilitate viral clearance.
- BLT mice model can be used to examine the mechanisms of immune defects during HIV infection and can be used for testing therapies aimed at reversing immune suppression.

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