Blockade of IFN-beta reactivates HIV reservoir in ART-suppressed HIV infected BLT Humanized Mice

531

Zhe Yuan[1], Manolis Papasavvas[1], Guorui Zu[1], Lily Lu[1], Matthew Fair[1], Samuel Keller[2], Luca Sardo[2], Guoxin Wu[2], Joel Cassel[1], Joseph Salvino[1], Paul Zuck[2], Claire Deleage[3], Bonnie J. Howell[2], Luis J. Montaner[1]
1.Wistar Institute, Philadelphia, PA, USA. 2. Merck & Co. Inc., Kenilworth, NJ, USA. 3. AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research. Frederick, MD, USA.

BACKGROUND

Despite the efficient suppression of HIV-1 replication can be achieved with combined antiretroviral therapy (cART), viral latency and low levels of type I interferon (IFN-I) signaling persist during chronic infection. This sustained signaling may promote T cell exhaustion and foster viral persistence. Using newly cloned human neutralizing antibodies specifically against IFNα or IFNβ, here we test the effects of selective blocking IFN-α or IFN-β in ART suppressed BLT humanized mice to determine the role each Type I IFN in suppression.

METHODS

Antibodies used for infusions against IFNα or IFNβ were characterized for activity by ELISA, STAT-1 phosphorylation, dimerization assay, and western blot to confirm specificity. Tissues and plasma from BLT humanized mice were used to measure IFNα and IFNβ expression as well as ISGs expression after HIV infection through qPCR, western, ELISA, IHC and RNAscope. BLT hu-mice were infected with transmitted/ founder (T/F) virus HIVsuma and following 4 to 5 weeks of viremia were suppressed on cART (FTC+TDF+RAL). Infusions of anti- IFNα/β-specific or IgG control antibodies were administered to the HIV fully suppressed animals intraperitoneally over three independent studies. Viral measures (plasma viral load, proviral DNA load, RNAscope in tissue), leukocyte subset activation, and metabolomics analysis with plasma. Analysis was done with Prism software.

Fig. 1

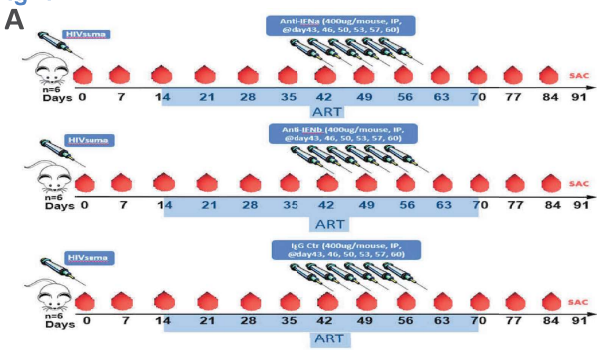

Figure 1. Study design. Blood drops indicated the bleeding time points; blue shades indicated the period of ART treatment.

Selective neutralization of human IFN-β but not IFN-α reactivated HIV-1 on ART in HIV-infected humanized mice.

RESULTS

Neutralizing anti-IFNα or anti-IFNβ-specific antibodies are capable of selectively blocking IFNα or IFNβ signaling in vitro and in vivo (data not shown). We found the infusions of anti-IFNβ but not IFNα or IgG control can repeatedly elicit repeated low level viremic blimps under ART (Fig 1 & 2A). We did not detect significant change in viral rebound after stopping ART between groups (Fig 2B). We confirmed IFNα and IFNβ expression in BLT humanized mice, and the increase in ISGs expression after HIV infection. And anti-IFN antibodies treatment can effectively decrease the ISGs expression (Fig. 2 C&D). HIV RNA per million CD4+ cells increased 24hrs post the IFNβ-specific antibody treatment (Fig 3A). The metabolomics analysis showed a significant metabolism shift with p value < 0.025 between IFN-β antibody treated and Ctr groups supporting increased energy metabolism, increased amino acids metabolism, increased taurine and thyroid hormone synthesis may related to increased viral protein synthesis and immune response post viral reactivation (Fig. 3 C&D).

Fig. 2

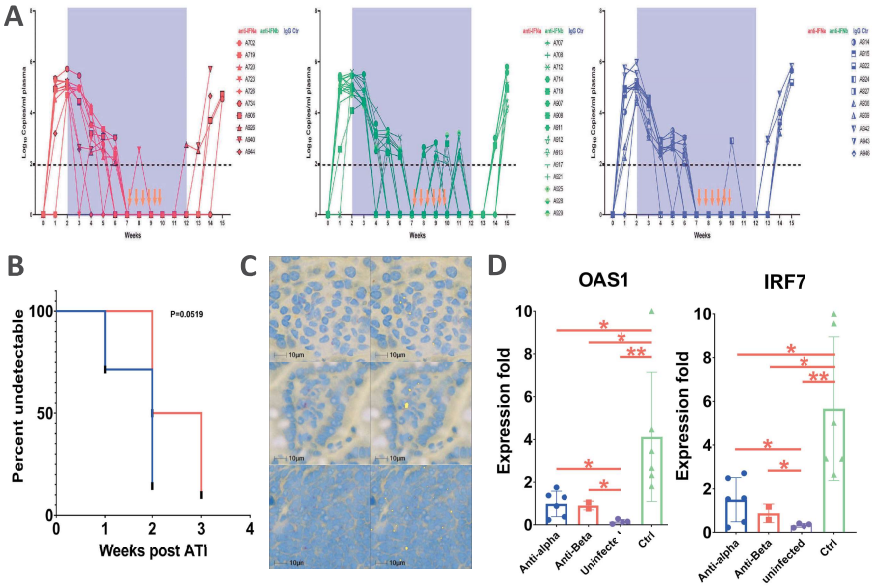

Figure 2. (A) Anti-IFNβ but not anti-IFNα or IgG control can repeatedly elicit repeated low level viremic blimps under ART. (B) Anti-IFNβ treatment did not delay the viral rebound post analytical treatment interruption (ATI). (C) RNAscope of IFN-β expression in humanized mice tissue: liver, gut and spleen (from top to bottom). Scale bar is 10um. (D) Anti-IFN antibodies treatment can effectively decrease the ISGs expression.

Fig. 3

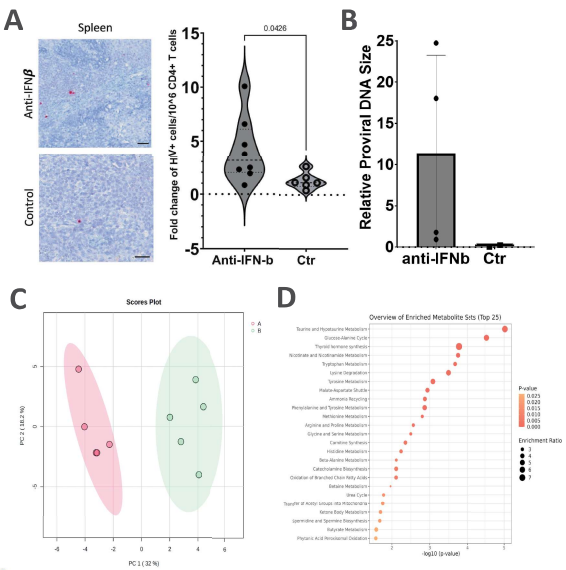

Figure 3. (A) HIV RNAscope signal in spleen of study animals and vRNA+ cells frequency 24 hrs post treatment. (B) HIV proviral DNA size 24hrs after the IFNβ-specific antibody treatment. (C) PCA plot of metabolomics analysis of IFNβ antibody treated HIV infected animals under ART. (D) Pathway enrichment analysis of plasma metabolites in IFNβ antibody treated HIV infected animals under ART.

CONCLUSIONS

Blockade of IFN-beta signaling can inhibit autologous negative regulation on cells infected by HIV resulting in reactivation of HIV expression on ART in HIV infected BLT humanized mice. The potential use of anti-IFNβ strategies together with direct anti-HIV "kill" or clearance strategies should be further investigated.

CONTACT AUTHOR

Luis J. Montaner montaner@wistar.org

ACKNOWLEDGEMENTS

This work was primarily funded by a grant by the NIH-funded BEAT-HIV Martin Delaney Collaboratory to cure HIV-1 infection (1UM1AI126620), Kean Family Professorship, the Philadelphia Foundation (Roberts I. Jacobs Fund).