Characterization of Interferon-α subtypes in the LPAC model

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Abstract

IFNα can inhibit acute HIV-1 infection but is also associated with pathogenesis. The mechanism for this dual impact of IFNα remains unknown. There are 12 IFNα proteins but only one, IFNα2, has been used clinically. Here we develop a method based on next-generation sequencing (NGS) to enumerate the relative abundance of different IFNα subtype transcripts. We then determined the relative potency of each IFNα subtype using the Lamina Propria Aggregate Culture model (LPAC), which recreates infection of the gut-associated lymphoid tissue (GALT), a major site of early HIV-1 replication.

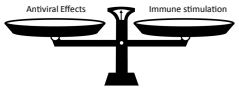
1. Expression of IFNα subtypes. Primer sets for quantitative PCR and Illumina NGS were designed in highly conserved regions that encompass polymorphic sites that allow for IFNα subtype classification. Plasmacytoid dendritic cells (pDCs), the major producers of IFNα, were negatively selected from PBMCs and exposed to cell-free R5-tropic HIV-1 (Ba-L) for 6 hours. pDCs showed IFNα induction of >400-fold after 6hrs post HIV-1 exposure. NGS analyses showed differential expression of IFNα subtypes in pDCs +/- HIV exposure. In particular, IFNα5, IFNα8, IFNα2, IFNα14 and IFNα1/13 were overrepresented with HIV exposure.

2. IFNα subtype potency in LPAC model. To determine the potencies of individual IFNα subtypes, each IFNα subtype was added to LPAC cells once at 100pg/mL immediately post-infection with HIV-Bal normalized to 10ng p24/mL. At 4dpi, IFNα8, IFNα6, and IFNα14 were the most potent in restricting productive HIV-1 infection and infectious virion release whereas IFNα1 was the least potent. The same differences in potencies were observed in 6 additional HIV-strains, with transmitted/founder virus clones significantly more resistant to IFNα than their paired chronic virus counterparts. The relative potencies were also reflected in the induction levels of four key restriction factors: A3G, BST-2, MX2, and Trim5. IFN inhibition resulted in a decrease in the infectivity of produced virions. These data indicate that the IFNα subtypes have qualitatively similar activities, and IFNα2 is not the most potent IFNα subtype for clinical use.

Introduction

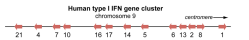
Interferon-α in HIV-1 Infection:

The key to using IFNα in a therapeutic or curative strategy is to separate the antiviral effects from the immune stimulatory effects. This can be done by finding and exploiting differences in activity of the IFNα subtypes, or by identifying key downstream antiviral mechanisms



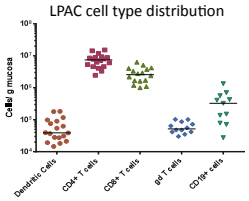
LPAC infection model:

The gut mucosa is an early site of HIV-1 replication and T cell depletion, when IFNα activity is most important to restriction. Cells harvested from the lamina propria of human jejuni contain the shown cell types, termed Lamina propria aggregate culture, and are naturally susceptible to HIV infection.



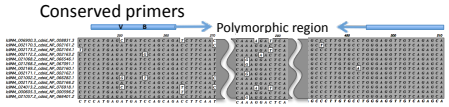
Interferon-α subtypes:

There are 12 individual IFNα proteins. While they all use the same receptor, it is poorly understood what differences may exist in their expression and potency.

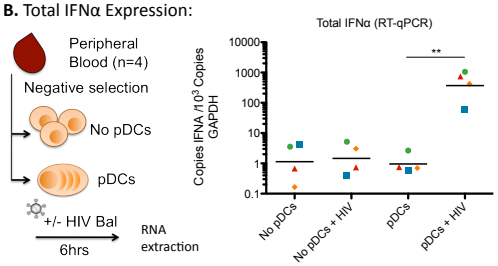


1. Expression of individual IFNα subtypes following HIV-1 exposure

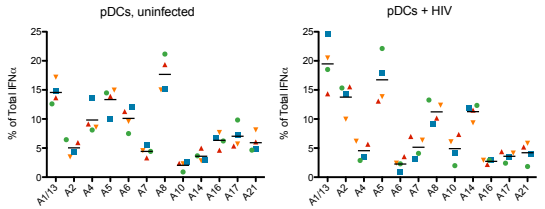
A. Primers Designed to amplify all IFNα subtypes simultaneously:



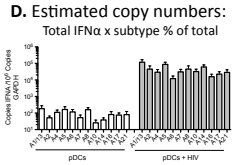
B. Total IFNα Expression:



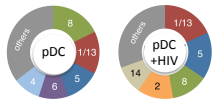
C. Distribution of IFNα-subtypes by Illumina sequencing:



D. Estimated copy numbers:

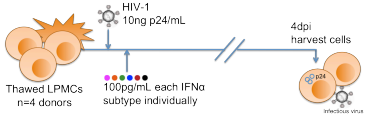


E. Subtype expression summary:

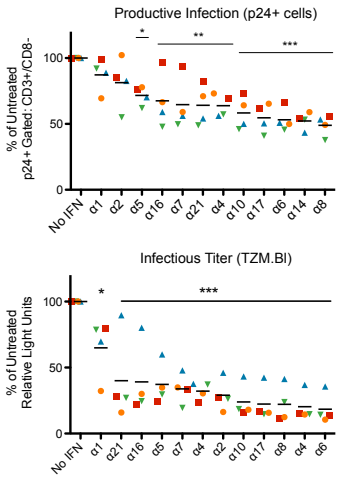


2. IFNα subtype potency against HIV-1 in LPAC model

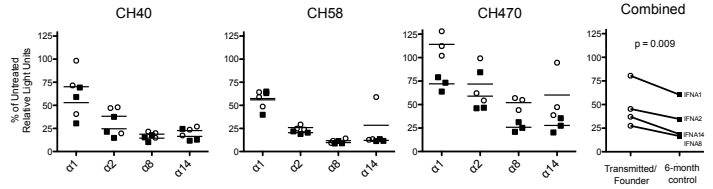
A. Experimental Design:



B. HIV-1 Ba-L inhibiton:

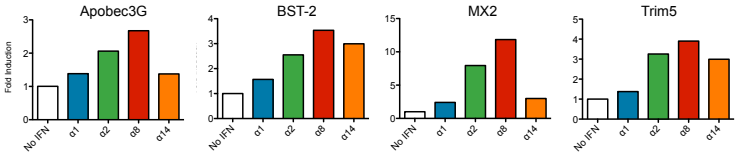


C. Transmitted founder (O) and 6-month control (■) infectious molecular clone inhibition:

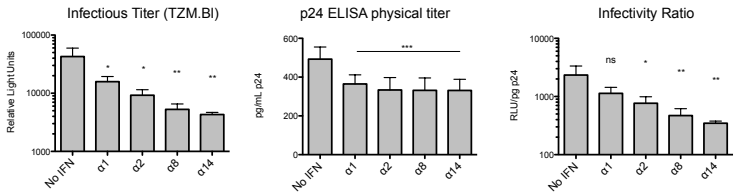


D. Interferon stimulated restriction factor expression:

LPAC infected with HIV-1, treated with IFNα, CD4+ T cells negatively selected 24hrs post treatment:



E. Non-infectious virus release:



Stats: A (n=4) C (n=3)donors. One-way ANOVA with Dunnett's post-test. * <0.05; **<0.01; ***<0.001

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