

# Type I Interferons Vary in Their Ability to Inhibit HIV Replication in Primary Macrophages

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F-158



## 1. ABSTRACT

Anti-HIV drugs must target cells of the macrophage lineage because they are one of the first cell types infected and provide a persistent reservoir for HIV replication. We aimed to characterize the ability of Type I interferons (IFNs) to protect monocyte-derived macrophages (MDMs) from HIV infection. Although all Type I IFNs assayed were able to inhibit HIV replication their ability to do so varied with the length of IFN treatment, the species of Type I IFN, and the concentration of IFN. Of those tested, IFN- $\alpha 2$  and - $\beta$  were the strongest inhibitors while low concentrations of weaker inhibitors may actually enhance HIV replication.

## 2. INTRODUCTION

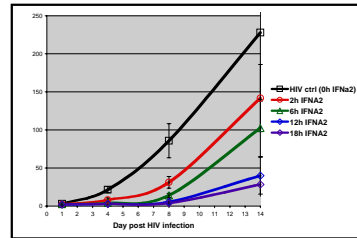
- IFN pathways and IFN stimulated genes (ISGs) contribute to innate immunity which provides the first line of defense against invading pathogens.
- The sequencing of the mammalian genome enabled the identification of all known species of Type I IFN: - $\alpha$  (n=13), - $\beta$ , - $\delta$ , - $\epsilon$ , - $\kappa$ , - $\tau$ , and - $\omega$ .
- Different species of Type I IFN vary in their ability to inhibit HIV replication in cell lines.
- IFN- $\alpha 2$  and IFN- $\beta$  can inhibit HIV replication in macrophages but other species of Type I IFN have not been characterized in primary cells.
- Although different Type I IFNs appear to vary in their anti-HIV activity, they have not been assessed in the same primary cell type.
- IFN stimulated genes: ISG15, OAS1, and EIF2AK2 (PKR), have been shown specifically to inhibit HIV replication.

## 3. METHODS

- Monocytes were isolated by adherence from PBMCs extracted from the blood of healthy donors and seeded in 48-well plates at a density which gave rise to  $2 \times 10^5$  MDMs per well after 5 days of MCSF treatment.
- For the majority of experiments MDMs were pretreated with IFN for 12 hours.
- Virus infections were performed with HIV Bal for 2 hours at an MOI of 0.3.
- HIV replication was assessed using an ELISA for p24 (Perkin Elmer).
- The statistical difference between IFN treatments was calculated by applying a one way analysis of variance (ANOVA) followed by a Tukey test for multiple pairwise comparison of p24 measurements.
- Quantification of OAS1 gene expression was determined by qRT-PCR using a 7900HT Real Time PCR System (Applied Biosystems).

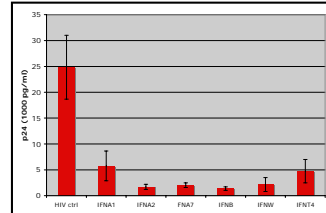
## 4. RESULTS

**Figure 1. The effect of varying the length of 186 pM (1000 IU) IFN- $\alpha 2$  pretreatment on HIV replication.**



- Macrophages were pretreated with 186 pM of IFN- $\alpha 2$  for different time periods and the effect on HIV replication assessed.
- An ANOVA Tukey test of p24 measurements showed that all pretreatment periods significantly inhibited HIV replication at Day 8.
- Longer pretreatment periods up to 12 hours were significantly better ( $p < 0.01$ ) at inhibiting HIV replication. There was no significant difference between 12 and 18 hours of pretreatment.

**Figure 2. The effect of other Type I IFNs on HIV replication.**



- Based on the results obtained in **Figure 1**, a 12 hour pretreatment of MDMs was selected to determine the ability of 186 pM concentrations of different Type I IFNs (- $\alpha 1$ , - $\alpha 2$ , - $\alpha 7$ , - $\beta$ , - $\tau$ , and - $\omega$ ) to inhibit HIV replication.
- An ANOVA Tukey test (**Table 1**) of p24 measurements at day 8 post HIV infection indicated that:
  - All IFN treatments significantly inhibited HIV replication ( $p < 0.01$ ).
  - all IFN treatments apart from IFN- $\tau$  were significantly better than - $\alpha 1$  ( $p < 0.01$ ).
  - only IFN- $\alpha 2$  and - $\beta$  were significantly better than - $\tau$  ( $p < 0.05$ ).
- Therefore, Type I IFNs could be classified as weak (- $\alpha 1$  & - $\tau$ ), moderate (- $\alpha 7$  & - $\omega$ ) and strong (- $\alpha 2$  & - $\beta$ ) inhibitors of HIV replication.

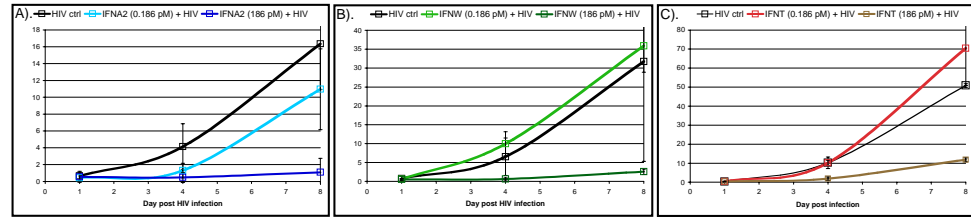
**Table 1. ANOVA Tukey test to assess the ability of different Type I IFNs to inhibit HIV replication.**

DAY8	IFNA1	IFNA2	IFNA7	IFNB	IFNT
IFNA1	0.002				
IFNA2	0.004	0.999			
IFNA7	0.001	0.999	0.981		
IFNB	0.876	0.032	0.096	0.013	
IFNT	0.006	0.997	1.000	0.959	0.989

## 5. CONCLUSIONS

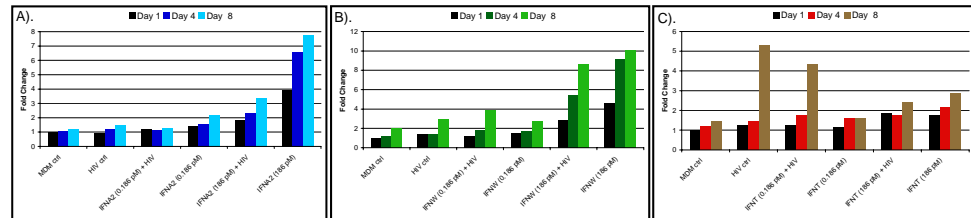
- As expected, longer pretreatments of Type I IFNs (>12 hours) at higher concentrations (186 pM) inhibit HIV replication in macrophages significantly better than shorter treatments (<12 hours) and lower concentrations (0.186 pM).
- All Type I IFNs inhibit HIV replication but can be further classified as weak (IFN- $\alpha 1$  & - $\tau$ ), moderate (IFN- $\alpha 7$  & - $\omega$ ) or strong inhibitors of HIV replication (IFN- $\alpha 2$  & - $\beta$ ).
- Low concentrations of weak and moderate inhibitors may actually sensitize macrophages for increased HIV replication. Since IFN- $\alpha 2$  is currently being evaluated for its ability to reduce viral load in HIV infected persons, this suggests that it is essential to maintain an effective concentration of this cytokine to prevent the possible sensitization of patient cells to the virus.
- Despite inhibition of HIV replication by 186 pM of IFN- $\alpha 2$  (**Figure 3A**), it appears that the virus can still downregulate anti-HIV ISGs like OAS1 (**Figure 4A**).
- The complete repertoire of important anti-HIV ISGs will be identified in the future using microarray gene expression analysis, which will identify ISGs that are upregulated by IFN treatment, downregulated by the virus following IFN treatment and not upregulated to a great extent by HIV alone.

**Figure 3. The effects of different concentrations of (A) IFN- $\alpha 2$ , (B) IFN- $\omega$ , and (C) IFN- $\tau$  on HIV replication.**



- The extent of HIV inhibition was investigated using 12 hr pretreatments of a strong (IFN- $\alpha 2$ ), moderate (IFN- $\omega$ ) and weak (IFN- $\tau$ ) inhibitor at two different concentrations, 0.186 and 186 pM.
- ANOVA Tukey tests of p24 measurements at day 8 suggested that in general 186 pM treatments of IFN were significantly better at inhibiting HIV replication compared to 0.186 pM treatments or the HIV control, which were no different from each other.
- Although not significant, low concentrations (0.186 pM) of weak (IFN- $\tau$ ) and moderate (IFN- $\omega$ ) inhibitors appear to sensitize macrophages to HIV replication such that p24 measurements for these conditions are greater than the HIV control.

**Figure 4. OAS1 gene expression following treatment with A) IFN- $\alpha 2$ , B) IFN- $\omega$ , and C) IFN- $\tau$ , in the presence or absence of HIV infection.**



- Gene expression of OAS1, an anti-HIV ISG, was evaluated by qRT-PCR at each time point and for each condition for the experiment performed in **Figure 3**.
- 186 pM of IFN- $\alpha 2$  and - $\omega$  lead to the greatest induction of OAS1 within these experiments, but for the IFN- $\tau$  experiment the greatest upregulation was evident in the HIV control.
- Strikingly, although **Figure 3A** indicates that 186 pM of IFN- $\alpha 2$  significantly inhibits HIV replication (dark blue line), it appears from **Figure 4A** that the virus is still capable of dramatically reducing OAS1 gene expression compared to the IFN- $\alpha 2$  only control.

## Acknowledgements

This work was supported by an R21 grant from the NIH (AI065242) and by the Genomics Core at the Center for AIDS Research San Diego (A136214).

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