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# Kinetics of HIV-1 Gene Expression Following Reactivation in a Primary Cell Model of Latency

Victoria Walker-Sperling[1], Valerie Cohen[2], and Joel Blankson[3]

[1] Immunology Graduate Program, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [2] Graduate Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[3] Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD, USA

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## Introduction

CD4+ T cells latently infected with HIV-1 pose a significant barrier to eradication. Proposed "shock and kill" strategies involve using small molecules to reactivate latent HIV-1 without causing the massive toxicity associated with non-specific T cell activation. While HAART will prevent new rounds of infection when latent virus is reactivated, the immune response must be capable of eliminating the few HIV-infected cells that will inevitably be left once treatment ceases. In order for CD8+ T cells to prevent new rounds of infection following reversal of latency, they must kill infected CD4+ T cells prior to the completion of the viral life cycle. Thus, we examined the kinetics of HIV-1 gene expression in a primary cell model of latency in the context of reactivation to determine the optimal time frame for CD8+ T cell mediated killing.

## Methodology

**Latently Infected Cells:** Resting CD4+ T cells isolated from HIV+ donors were stimulated with PMA/ionomycin for 6 hours in the presence of RAL and EFV. HIV mRNA from culture supernatant sampled at 6 and 24 hours and cell lysate at 24 hours was quantified via qPCR.

**Nucleofection:** Resting CD4+ T cells from HIV- donors were nucleofected with either $HIV_{NL4\text{-}3\Delta Env\ GFP}$ or $HIV_{NL4\text{-}3\Delta Nef\ GFP}$ and stimulated with anti-CD3/CD28 Dynabeads for up to six hours. At various timepoints, cells were examined for expression of CD4, MHC-I, GFP, and Gag by flow cytometry (FACS Canto II).

## Results

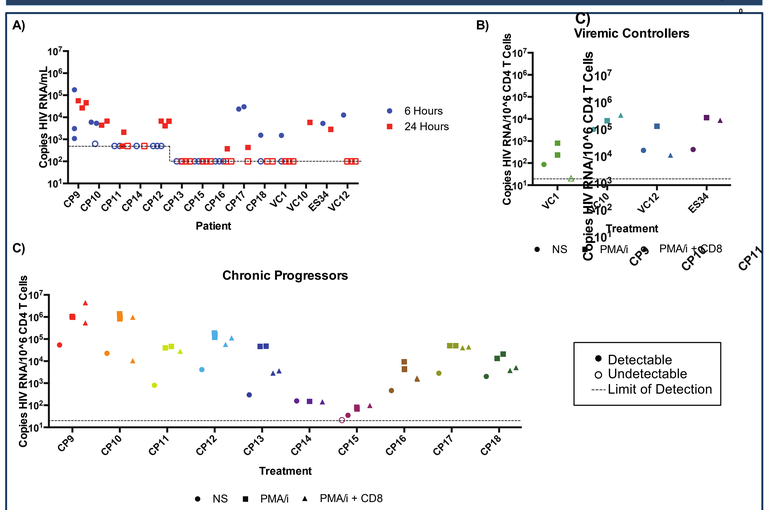



**Figure 1. Reactivated, latently-infected resting CD4+ T cells produce virions as early as 6 hours, although Gag-stimulated CD8+ T cells do not appear to have much effect in inhibiting production.** Resting CD4+ T cells from HIV+ donors were stimulated with PMA/ionomycin for 6 hours, at which point the media was replaced and autologous CD8+ T cells stimulated with overlapping Gag peptides for 7 days were added to cultures. RNA isolated via Trizol extraction from supernatant and cell lysates was quantified with qPCR measuring HIV mRNA transcripts. A) Supernatant transcript levels from PMA/ionomycin-treated cells. B) Intracellular transcript levels in viremic controllers (24 hours). C) Intracellular transcript levels in chronic progressors (24 hours).

## Results

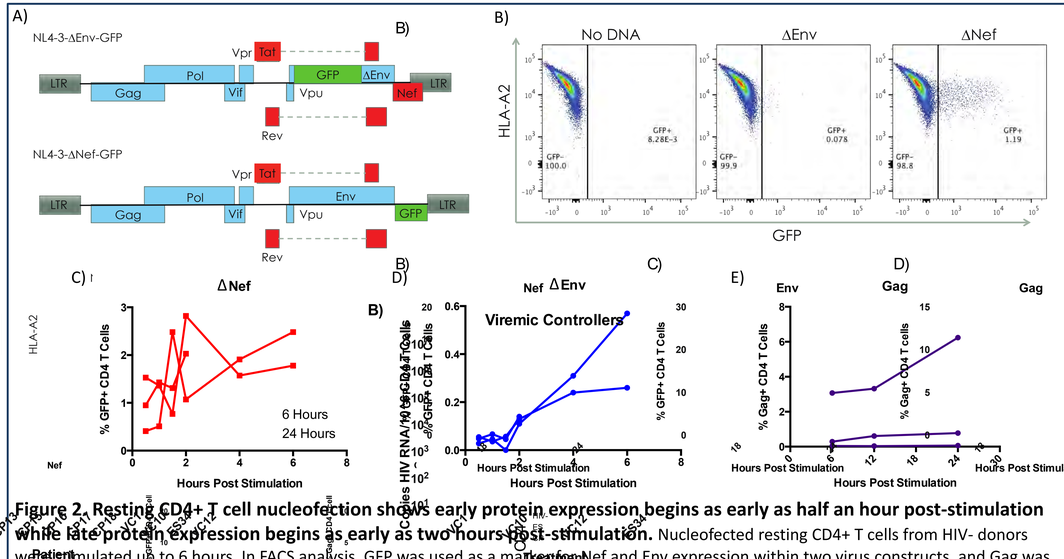



**Figure 2. Resting CD4+ T cell nucleofection shows early protein expression begins as early as half an hour post-stimulation while late protein expression begins as early as two hours post-stimulation.** Nucleofected resting CD4+ T cells from HIV- donors were stimulated up to 6 hours. In FACS analysis, GFP was used as a marker for Nef and Env expression within two virus constructs, and Gag was measured via antibody. A) HIV-$1_{NL4\text{-}3}$ variants nucleofected into resting CD4+ T cells. B) FACS gating for nucleofected cells (example from 6 hours post-stimulation shown). C) GFP expression with the ΔNef-GFP construct. D) GFP expression with the ΔEnv-GFP construct. E) Gag expression seen with the ΔEnv-GFP construct.

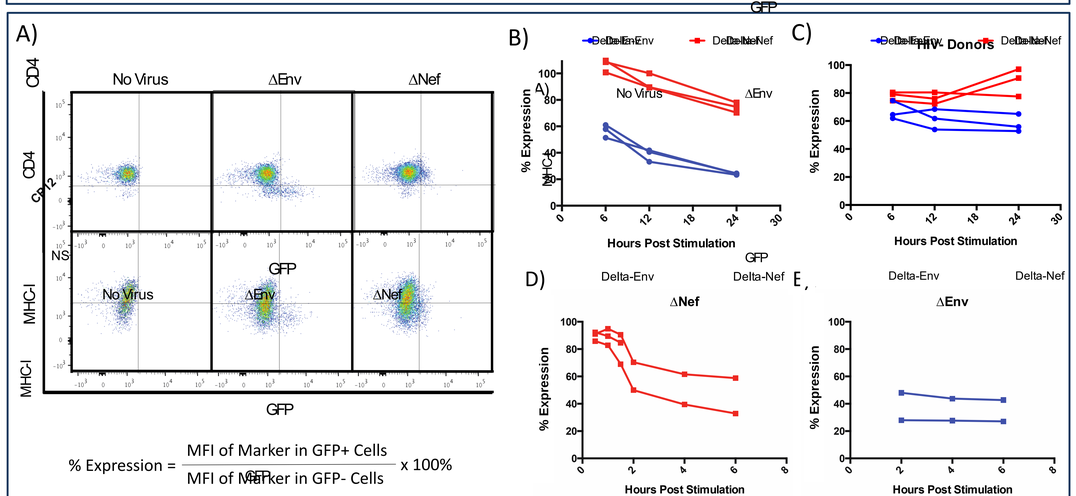



**Figure 3. Downregulation of CD4 begins as early as six hours post-stimulation and downregulation of MHC-I begins as early as 1.5 hours post-stimulation.** A) FACS diagram of CD4 and MHC-I downregulation (representative data from 12 hours post-stimulation) and equation for % Expression, which is calculated with the mean fluorescence intensity (MFI) of either CD4 or MHC-I in the GFP+ and GFP- cell populations. B) Expression of CD4 in GFP+ cells nucleofected with $HIV_{NL4\text{-}3\Delta Env\ GFP}$ or $HIV_{NL4\text{-}3\Delta Nef\ GFP}$. C) Expression of MHC-I in GFP+ cells nucleofected with $HIV_{NL4\text{-}3\Delta Env\ GFP}$ or $HIV_{NL4\text{-}3\Delta Nef\ GFP}$. D,E) Early expression of HLA-A2 in GFP+ cells nucleofected with ΔNef-GFP (D) or ΔEnv-GFP(E).

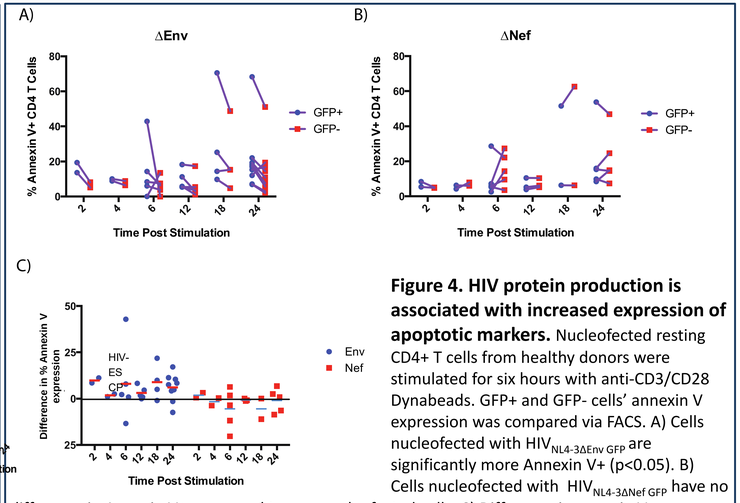
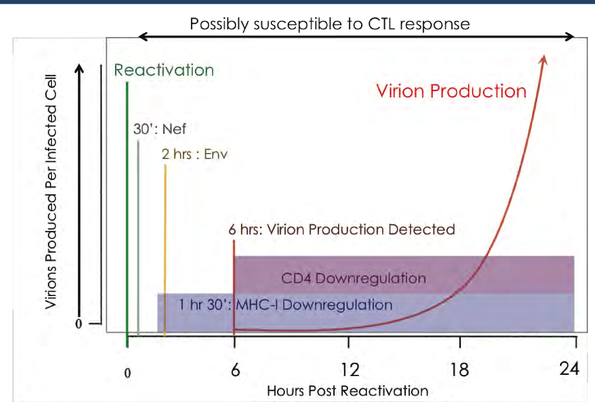



**Figure 4. HIV protein production is associated with increased expression of apoptotic markers.** Nucleofected resting CD4+ T cells from healthy donors were stimulated for six hours with anti-CD3/CD28 Dynabeads. GFP+ and GFP- cells' annexin V expression was compared via FACS. A) Cells nucleofected with $HIV_{NL4\text{-}3\Delta Env\ GFP}$ are significantly more Annexin V+ ($p<0.05$). B) Cells nucleofected with $HIV_{NL4\text{-}3\Delta Nef\ GFP}$ have no difference in Annexin V+ compared to non-nucleofected cells. C) Difference in annexin V expression between GFP+ and GFP- cells.

## Conclusions




- Virions are produced by latently-infected CD4+ T cells as early as six-hours post PMA/ionomycin treatment in some patients.
- Early protein (Nef) was produced as soon as 0.5 hours and late protein (Env) by two hours after activation of nucleofected CD4+ T cells.
- Downregulation of MHC-I begins even in the absence of Nef 1.5 hours after stimulation of nucleofected CD4+ T cells.
- Stimulated CD8+ T cells from some viremic controllers amd chronic progressors were capable of suppression of HIV mRNA production in CD4+ T cells 24 hours post-stimulation.
- These results suggest that it may be possible to eliminate recently reactivated, latently-infected cells before new rounds of viral replication occur.