**Viral Kinetics: Exploring HIV-1 Infection in Endothelial Co-cultured CD4+ Resting T Cells**

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**Abstract**

Resting CD4+ T cells cannot normally be successfully infected in vitro with HIV-1. Co-culture of resting CD4+ T cells with endothelial cells (ECs) has been shown to boost the normally low rates of infection to rates close to or exceeding those of activated CD4+ T cells. Latency is a primary focus of HIV research and understanding infection dynamics and kinetics of different cell types is a crucial first step to understanding latency formation in these cells. The kinetics of the EC co-cultured resting CD4+ T cells had not previously been studied and so we set out to compare the infection kinetics of the co-cultures of those of resting and activated CD4+ T cells cultured alone. Through the use of drugs to block reverse transcription and integration on different days post infection, the timeline of HIV-1 viral infection in the EC co-cultures was examined. Both integration and reverse transcription occurs at a significantly slower rate for EC co-cultures and resting cells compared to the activated CD4+ T cells. For reverse transcription we found that speed of EC stimulated cells showed no difference from resting cells cultured alone. For integration, resting and EC co-cultured cells showed no difference from each other, while EC+ (EC stimulated with IFN-g) co-cultures were slightly faster. The results demonstrated that even though EC co-culture significantly increases infection rates in resting T cells, it does not change viral kinetics in these cells.

**Materials and Methods**

**Virus Preparation:** A pseudo-typed virus was created by transfecting pNL43dE-GFP with a plasmid encoding an HIV env utilizing CCR5.

**T Cell Isolation and Co-culture:** Resting and Total CD4+ T cells were isolated using Miltenyi MACS beads from HIV negative PBMC. Total CD4+ cells were activated with anti-CD3 and CD28 antibodies two days prior to virus infection. For the co-cultures, ECs with (EC+) or without (EC-) IFN-g stimulation were plated and resting T cells were added.

**One day later,** resting T cells alone, CD3/CD28 activated T cells and the EC-/EC+ co-cultured T cells were infected with the pseudo-typed virus.

**Drugs:** The reverse transcriptase inhibitor Efavirenz (100 nM) and the integrase inhibitor Raltegravir (3.3 uM) were used to block infection at day 0 (1hr prior to infection), 12hrs and days 1-5 post infection. Raltegravir was refreshed every other day, and Efavirenz was refreshed every fourth day to insure continued blockage of viral life cycle.

**Infection Rates:** Infection rates on day six were determined by % GFP using the flow cytometer. The percent total infection was calculated by comparing %GFP on various days to the average of Day 6 values (no drugs added).

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**Results and Discussion**

1. **Reverse Transcription:** The results demonstrated that even though EC co-culture significantly increases infection rates in resting T cells, it did not change viral kinetics in these cells.

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**References**
