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EFFECTS OF DIFFERENTIAL CELL SIGNALING IN HIV TRANSCRIPTION

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ABSTRACT

Background: After cessation of antiviral treatments, HIV viral levels rebound due to the existence of latent reservoirs. The elucidation of signals that bias a provirus towards a latent or robust infection is necessary for more thorough treatment of HIV/AIDS patients. We propose a model system by which signals from CD3 and CD28, receptors necessary for T cell activation, can be manipulated at the time of infection of CD4⁺ T cells. This allows us to alter the balance between productive infection and the establishment of latent reservoirs.

Methods: Lentiviral vectors were used to transduce Jurkat and primary T cells with chimeric antigen receptors, which act as surrogates for the T cell receptor upon engagement with the corresponding ligand. These receptors include intracellular CD28 and CD3-zeta signaling domains fused to one of several anti-Her2 scFvs displaying different quantifiable affinities for Her2. Flow cytometry was used to isolate transduced cells. Expression of HIV was measured via luciferase or GFP. Integration of HIV was measured via nested Alu RT-PCR.

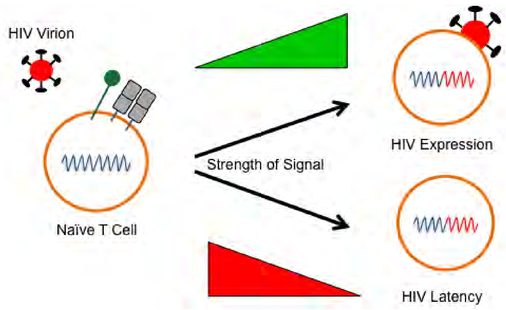
Results: Receptors were successfully transduced into CD4⁺ T cells. Preliminary data indicates that CAR activation leads to a more than 3 fold increase in HIV expression. Receptor affinity for Her2 does not correlate linearly with the level of HIV expression, indicating that signaling thresholds must be met for robust proviral transcription.

Conclusions: Adjusting the intensity of cellular signals at the time of infection allows us to control the robustness of HIV transcription and the course of the viral infection.

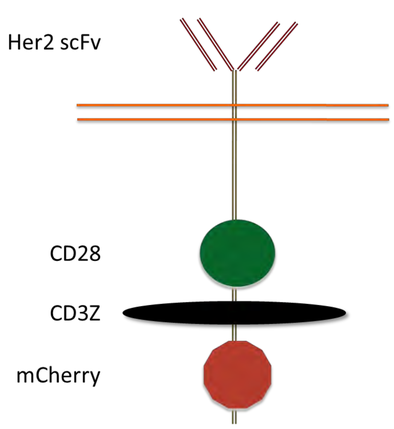
BACKGROUND

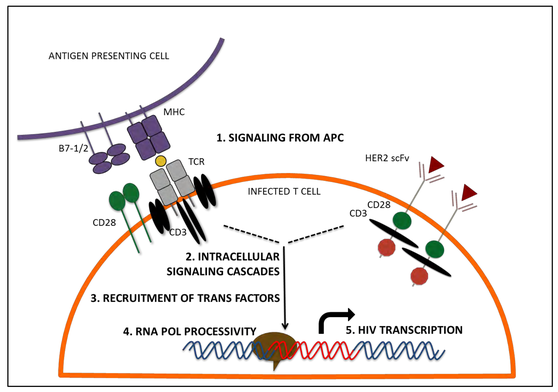
HIV Latency

HYPOTHESIS: The dynamics of T cell receptor signaling at the time of HIV infection determines the robustness of proviral transcription and the potential for latent infection.



Chimeric Antigen Receptors as Model System to Analyze T Cell Receptor Signaling





RESULTS

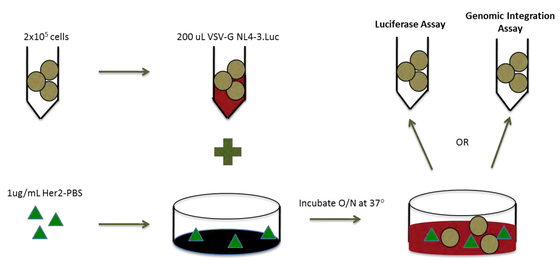
Differential Affinities of Her2 scFvs

Receptor	K_d (M)
C6-***B1D2***	1.5×10^{-11}
C6M***H3-B1***	1.2×10^{-10}
C6M***L3-9***	1.0×10^{-9}
C6.5	1.6×10^{-8}
C6.5***G98A***	3.2×10^{-7}

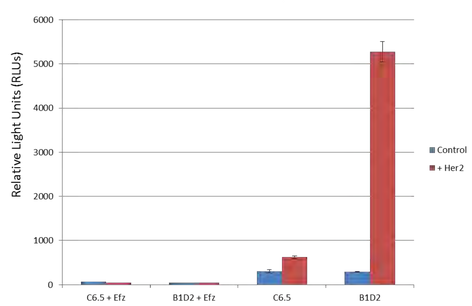
Experimental Strategy

A spinoculation was used to transduce Jurkat T cells with the various CARs in pHR lentiviral vectors. We sorted the transduced cells on a MoFlo Legacy Machine by gating for mCherry positive cells. Two months after sorting, stable expression of the chimeric antigen receptors was confirmed with LSRII.

Her2 Stimulation at the Time of Infection

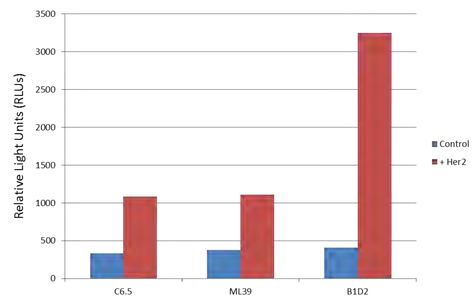


Increased T Cell - Associated Signals Upregulate HIV Transcription



After activation of mCherry⁺ Jurkat T cells at the time of infection with VSV-G pseudotyped NL4-3. Luc, cells were incubated overnight at 37°. The following day, cells were lysed and luciferase values measured with a luminometer. Values from one representative experiment (in triplicate) were averaged. Controls indicate wells coated with 5% FBS-PBS in place of 1 ug/mL Her2. Efz indicates HIV + Efavirenz (NNRTI).

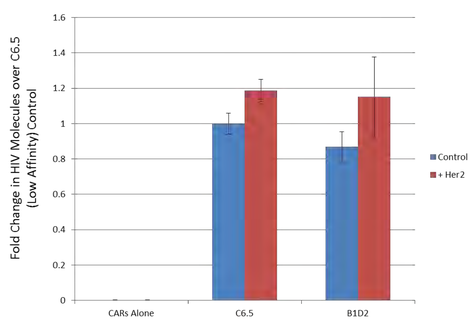
Signaling Thresholds Exist for Maximal HIV Transcription



After activation of mCherry⁺ Jurkat T cells at the time of infection with VSV-G pseudotyped NL4-3. Luc, cells were incubated overnight at 37°. The following day, cells were lysed and luciferase values measured with a luminometer. Values are presented from one preliminary experiment. Controls indicate wells coated with 5% FBS-PBS in place of 1 ug/mL Her2.

Nested Alu-PCR For Integration Analysis

T Cell Stimulation Does Not Affect HIV Integration

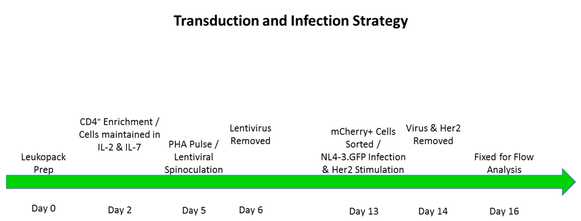


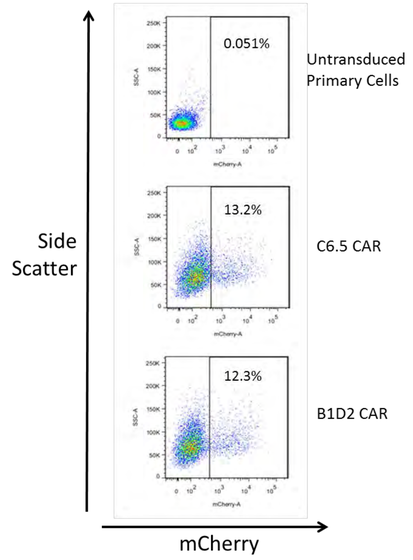
Using a Nested Alu-PCR strategy, integrated HIV DNA was measured and compared with a pNL4-3 copy standard. Values from one representative experiment (in triplicate) were averaged and normalized to the amount of amplified HIV molecules in the C6.5 Control (the low affinity cell-line without Her2 stimulation at the time of HIV infection). Controls indicate wells coated with 5% FBS-PBS in place of 1 ug/mL Her2. CARs Alone indicates mCherry⁺ cells without virus as a confirmation that the nested PCR strategy does not amplify the lentiviral CAR-containing vector.

Transduction of CARs into Primary Cells

Primary CD4⁺ T cells were isolated from a donor leukapheresis pack. Cells were pulsed with phytohemagglutinin (PHA) prior to spinoculation with either the C6.5 or B1D2 – containing pHR lentiviral vector. Cells were given sufficient time to return to a resting state as measured by CD65 and CD29 expression. Cells were then sorted on a MoFlo Legacy for mCherry expression prior to Her2 stimulation at the time of NL4-3.GFP infection. Three days post infection, cells were fixed for analysis with LSRII.

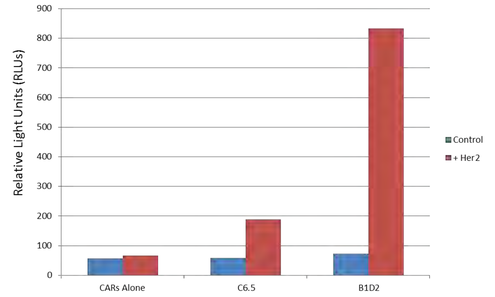
Transduction and Infection Strategy





Cells fixed 3 days post – transduction.

Increased T Cell - Associated Signals Upregulate HIV Transcription in Primary Cells



Primary cells were transduced according to scheme presented in above figure. On day 11 after leukapheresis pack preparation, cells were infected with VSV-G pseudotyped NL4-3.Luc. The following days, cells were washed and maintained at 37° in RPMI + IL-2 & IL-7 until day 15. Cells were lysed and luciferase values measured with a luminometer. Values are presented from one preliminary experiment. Controls indicate wells coated with 5% FBS-PBS in place of 1 ug/mL Her2. CARs Alone indicates mCherry⁺ cells without virus.

CONCLUSIONS

- We have successfully demonstrated the use of a Jurkat and primary CD4⁺ T cell model to analyze the effects of modulating T cell receptor signaling at the time of HIV infection.
- T cell stimulation through the engineered chimeric antigen receptor upregulates HIV expression at early time points.
- Preliminary data indicates that T cell receptor signaling does not correlate linearly with HIV transcription, indicating the existence of signaling thresholds
- T cell stimulation and the expression of different CARs do not alter HIV integration
- Future work will include the use of ChIP to measure RNAPII processivity and the abundance of transcription factors at the HIV LTR. Site –directed mutagenesis will also enable us to further analyze the impact of CD3ζ and CD28 on HIV transcription.
- Experiments are currently being undertaken to correlate initial T cell – associated signaling with the potential for HIV reactivation.

ACKNOWLEDGEMENTS

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