Fatty Acid Synthesis Controls Both Cellular Susceptibility to HIV and New Particle Production

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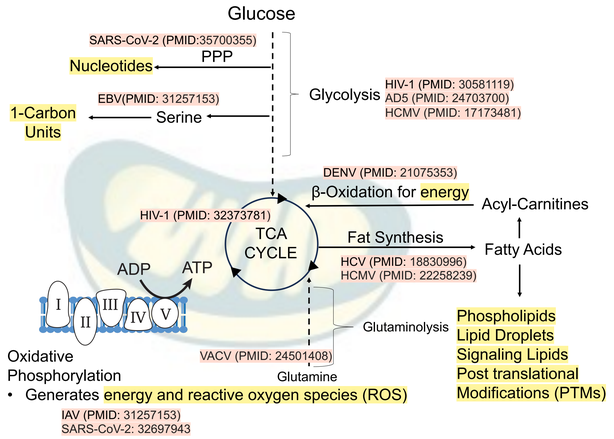
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320

BACKGROUND

Viruses are obligate intracellular pathogens that must source metabolites from infected hosts



The metabolome is a virus: host interface

HYPOTHESIS

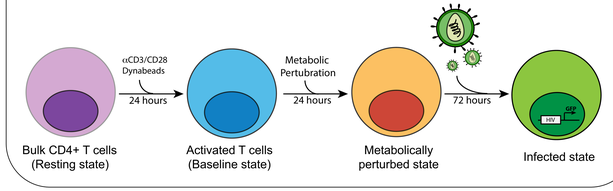
States of metabolic stress shape the virus: host interface, influencing the dynamics of viral infections.

METHODS

This study utilizes:

1. Primary CD4+ T cells from healthy blood donors
2. CEM cells as an *in vitro* model of HIV-1 infection
3. Single-cycle, VSV-G pseudotyped NL43 to assess first round infections as a correlate of susceptibility

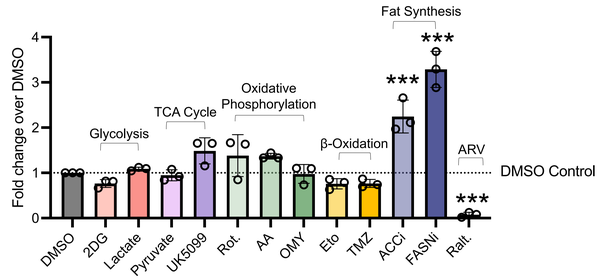
All perturbational studies are designed as follows



Key Message: Blocking cellular fat synthesis enhances the susceptibility of CD4+ T cells to HIV-1 infection, while decreasing new viral particle production. This is reversible with exogenous fatty acids.

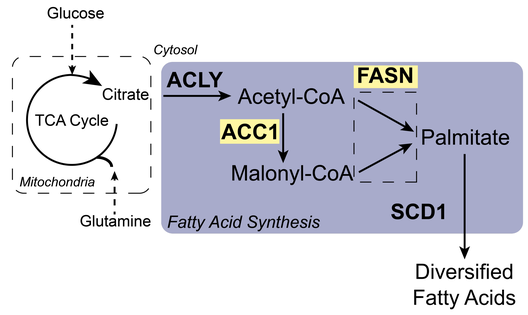
RESULTS

1. Metabolically focused small molecule screening implicates fat synthesis in CD4+ T cell susceptibility to HIV-1



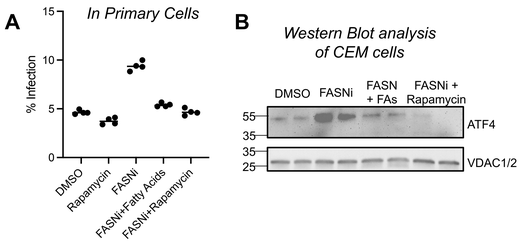
Metabolic inhibitor mini-screen to identify states of cellular susceptibility or resistance to HIV-1
Acutely activated CD4+ T cells were treated with the listed metabolic inhibitors for 24 hours prior to infection to screen metabolic pathways that shape susceptibility to infection. Each dot represents the average of two technical replicates for each donor (n=3), normalized to the DMSO treated controls. Significance was determined by one-way ANOVA and Holm Sidak's multiple comparison test.

2. Overview of Fatty Acid Synthesis



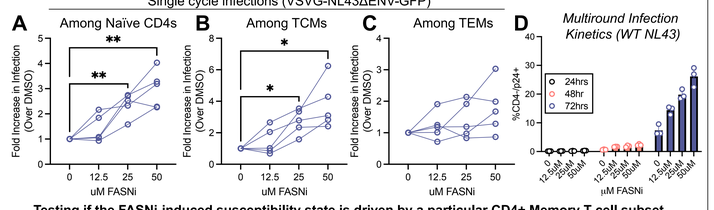
Enzymes and metabolic intermediates in the synthesis of fatty acids from citrate
Mammalian cells synthesize the 16-carbon saturated fatty acid palmitate from TCA cycle derived citrate and then diversify this fatty acid through elongation and desaturation. The two central enzymatic steps in this process that were identified by drug screen are highlighted in yellow.

6. Susceptibility and ATF4 expression depend on mTOR signaling



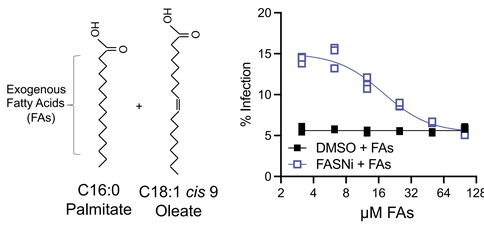
Testing the role of mTOR signaling in the susceptibility state
CD4+ T cells were primed with either rapamycin alone (mTORC1 inhibitor), FASNi, FASNi with exogenous FAs, or FASNi with rapamycin and susceptibility was assessed. Each dot represents technical replicates for one representative donor (A). Western blot on CEMs treated with either FASNi, FASNi with exogenous Fatty acids (FAs) or FASNi with rapamycin demonstrating a dependency of ATF4 induction on mTORC1 signaling during the inhibition of cellular fat synthesis. Each condition consists of two experimental replicates. (B)

3. Blocking fat synthesis enhances susceptibility in naïve and central memory (TCM) CD4+ T cells



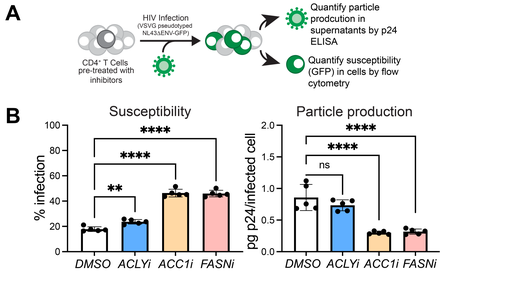
Testing if the FASNi-induced susceptibility state is driven by a particular CD4+ Memory T cell subset
Analysis of susceptibility to infection in different CD4+ T cell memory subsets based on CD62L and CD45RA staining (A-C). Each dot represents the average of three technical replicates for each donor (n=5). Significance was determined by one-way ANOVA and Holm Sidak's multiple comparison test. Analysis of susceptibility in bulk CD4+ T cells with replication competent NL43 (D). One representative donor is shown in technical triplicate.

4. This susceptibility state is reversible with exogenous fatty acids (FAs)



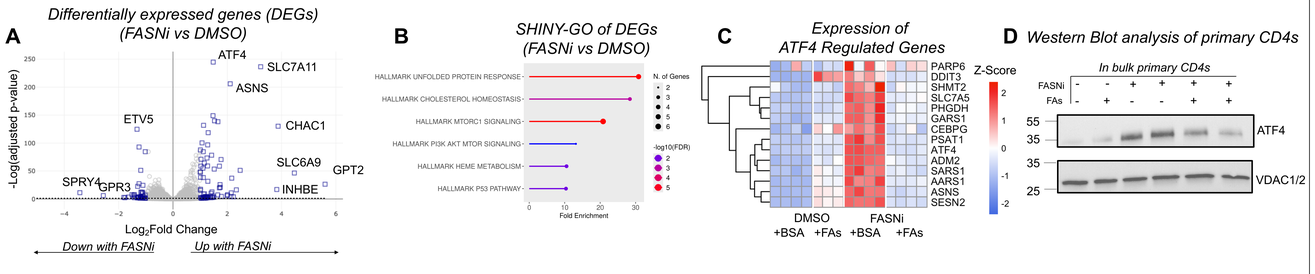
Can the end-product of fatty acid synthesis rescue this phenotype?
Activated CD4+ T cells were primed with either FASNi (50μM) or DMSO in the presence of a dose-escalation of BSA-conjugated fatty acids (C16:0 Palmitate and C18:1 Oleate) prior to infection. One representative donor is shown, with three technical replicates.

7. Inhibiting fat synthesis enhances susceptibility while decreasing new particle production



Does blocking fatty acid synthesis impact particle production?
CEM cells were primed with chemical inhibitors against the sequential steps of fat synthesis or DMSO as a vehicle control prior to infection. Susceptibility was measured based on the percentage of cells with GFP expression, while supernatants from matched cultures were collected for p24 ELISA to quantify viral particle production (schema shown in A). Each dot represents an experimental replicate. Statistics were determined by one-way ANOVA with Holm-Sidak's multiple comparison test.

5. ATF4 is induced in the susceptibility state, and its expression is reversed with the addition of exogenous fatty acids



Bulk RNA sequencing of the susceptibility state to uncover mechanistic drivers of susceptibility
CEM cells were primed with either DMSO or FASNi for 24 hours in the presence of exogenous BSA-conjugated fatty acids or BSA as a vehicle control (n=4 per group; n=3 for DMSO + FAs). Cells were washed, and total RNA was isolated. Bulk RNA sequencing was conducted with Azenta. (A) Transcript counts were normalized in DESEQ2, and a model was generated consisting of FASNi treatment, FA treatment, and an interaction term between FASNi and exogenous FAs. Differentially expressed genes were identified and represented as a volcano plot with statistical cutoffs as indicated by color. (B) SHINY-GO utilizing the Hallmark gene-set collections was conducted on differentially expressed genes (DEGs), and the most enriched pathways are shown. (C) Heatmap showing the z-scored expression of known ATF4-regulated genes across treatment groups. (D) Western blot analysis on primary CD4+ T cells that were primed with either FASNi or DMSO, and with the addition of exogenous fatty acids or BSA vehicle control. ATF4 expression is shown with VDAC to demonstrate equivalent protein loading between lanes.

Conclusions and Future Directions

Inducing fat stress in CD4+ T cells enhances susceptibility to HIV-1 while decreasing the generation of new viral particles.

1. Does ATF4 directly establish the susceptibility state?
2. Do other stimuli of ATF4 expression confer susceptibility?
3. How do cells sense alterations in fat synthesis and propagate this signal to ATF4?
4. Can inhibitors of fat synthesis synergize with ART to reduce residual viremia in people living with HIV?

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For further questions, please contact us at jacklin@mgh.harvard.edu