Retinoic Acid Blunts the Aryl Hydrocarbon Receptor/SAMHD1-Dependent HIV-1 Restriction in Monocyte-Derived Macrophages

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BACKGROUND

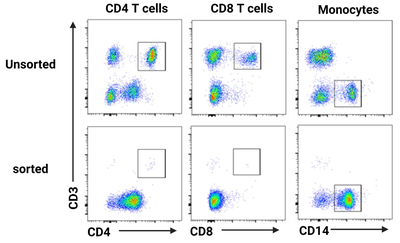
As part of the cell-autonomous innate immune system, SAMHD1 restricts HIV-1 replication by limiting the pool of deoxyribonucleoside triphosphate required for efficient reverse transcription. The antiviral activity of SAMHD1 is abrogated by phosphorylation. Among regulators of SAMHD1 activity, ligands of aryl hydrocarbon receptor (AhR) were reported to decrease SAMHD1 phosphorylation *via* mechanisms involving the transcriptional repression of kinases CDK1/2 (1) . Conversely, we demonstrated that retinoic acid (RA) promotes HIV-1 replication in T cells and macrophages (2-3). RNA sequencing analyses of *all-trans* retinoic acid (ATRA)-treated MDMs show that several gene signatures of AhR activation are differentially modulated, while cyclin-related proteins are upregulated (3). Considering the role of macrophages in HIV-1 infection in anatomic sites rich in both AhR ligands and RA, such as the intestine, here we investigated how the crosstalk between these two pathways governs SAMHD1 activity and HIV-1 replication in macrophages.

HYPOTHESIS:

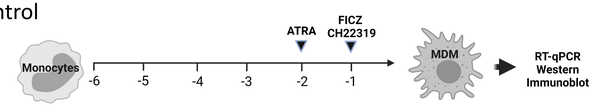
ATRA interferes with the HIV-1 antiviral activity of AhR activation through the inactivation of SAMHD1

METHODS

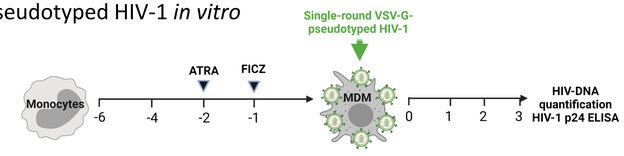
1. Enrichment of monocytes from HIV- PBMCs by negative selection using magnetic beads (Miltenyi)



2. Differentiation of monocyte-derived macrophages (MDMs) using M-CSF and treatments with ATRA, FICZ/CH22, and vehicle control



3. Infection of ATRA/FICZ-treated MDMs with single-round VSV-G pseudotyped HIV-1 *in vitro*



SUMMARY

- AhR inhibits HIV-1 reverse transcription in macrophages by decreasing the phosphorylation of SAMHD1
- ATRA reverses the antiviral effect of AhR by
  - ↓↓↓ ARNT, essential for AhR nuclear localization
  - ↑↑↑ AHRR, a repressor of AhR transcription
  - ↑↑↑ CDK1, involved in SAMHD1 phosphorylation

RESULTS

Figure 1: ATRA-treated MDMs downregulate transcripts associated with AhR signaling

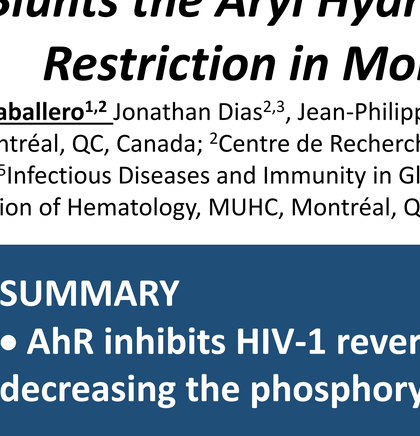


Figure 2: ATRA blocks the antiviral effect of FICZ

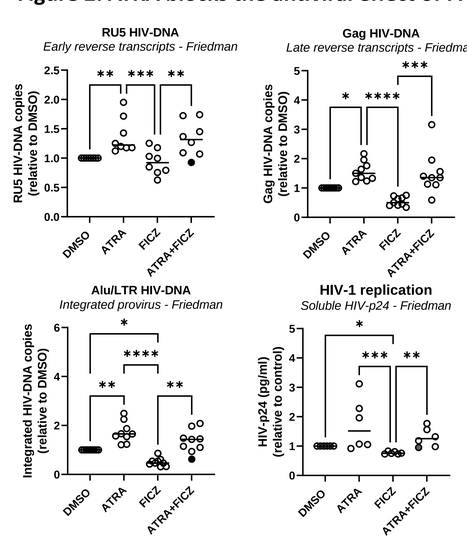


Figure 3. ATRA reverses the inhibitory effect of FICZ on CDK1 expression

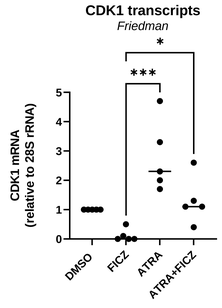
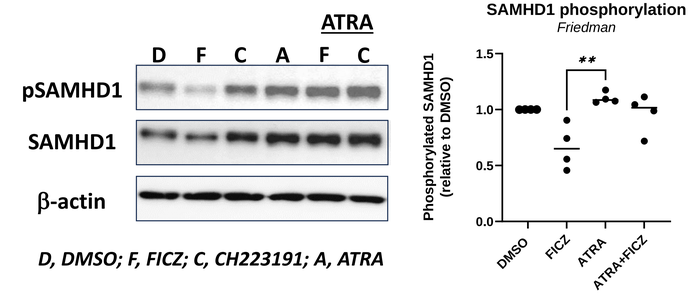


Figure 4. ATRA induces the phosphorylation of SAMHD1 in the presence or the absence of FICZ



CONCLUSIONS

- FICZ inhibits the HIV-1 replication cycle in MDMs, as revealed by decreased levels of early and late reverse transcripts, integrated HIV-DNA, and virion release in cell culture supernatants, while ATRA treatment shows proviral effect.
- ATRA interferes with AhR signaling via the downregulation of the AhR nuclear transporter ARNT, and the upregulation of the AhR repressor (AHRR). Together these results suggest that under the influence of ATRA, AhR signaling is inhibited resulting to a blunted antiviral response in MDMs
- CDK1 is responsible for the phosphorylation of SAMHD1, with phosphorylated SAMHD1 being ineffective in limiting HIV-1 replication. CDK1 is downregulated in the presence of FICZ, while its expression is upregulated in the presence of ATRA. Interestingly, in the presence of FICZ, ATRA is still capable inducing phosphorylation of SAMHD1 to promote HIV-1 replication in MDMs.
- Insulin-induced gene 1 (INSIG1) is upregulated in FICZ-treated MDMs (1) and is capable of limiting HIV-1 production through degradation of Gag (4). Interestingly, ATRA downregulates INSIG1. This suggests another mechanism by which ATRA overrides the antiviral effect of AhR activation in MDMs.
- Our results demonstrate opposing roles for AhR and RA pathways in the modulation of the antiviral activity of SAMHD1 and reveal a blunted AhR-mediated antiviral program in MDMs in the presence of RA. These studies identify the need for natural SAMHD1 modulators as novel therapeutic targets for HIV-1.

REFERENCES

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