Activation of Latent HIV-1 by Retinol Binding Protein 4

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Retinol Binding Protein 4, the carrier of Vitamin A in human blood, efficiently **activates latent HIV-1** proviruses via the **JAK/STAT5** and **JNK** signaling

BACKGROUND

It is currently poorly understood which endogenous factors reactivate HIV from latency *in vivo*

AIM

Identification of natural activators of latent HIV-1

METHODS

Screening of a human hemofiltrate derived peptide/ protein library for novel latency reactivators in J-Lat cells

Measurement of viral protein induction in cART-suppressed PBMCs through HIV-1 Gag-p24 Simoa

Functional analyses to discover the underlying mechanisms

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Identification of RBP4 as activator of latent HIV-1 proviruses

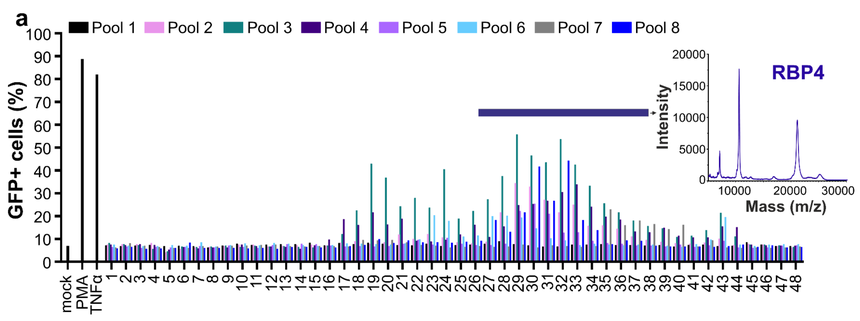


Figure 1. (Left) Activation of latent HIV-1 eGFP reporter proviruses in J-Lat 11.1 cells treated with peptide fractions of a hemofiltrate (HF)-derived library. (Right) MALDI-TOF of the active fractions identify **RBP4**

RBP4 activates latent HIV-1 in a variety of T cell lines

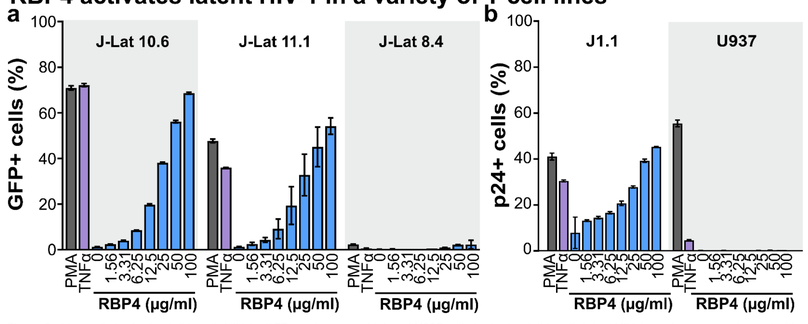


Figure 2. J-Lat cells harboring full-length HIV-1 eGFP proviruses or J1.1 and U937 cells harboring full-length intact HIV-1 proviruses were treated with RBP4, PMA or TNFα and the % of GFP+ cells were determined by flow cytometry.

Retinol is required but not sufficient for HIV-1 reactivation

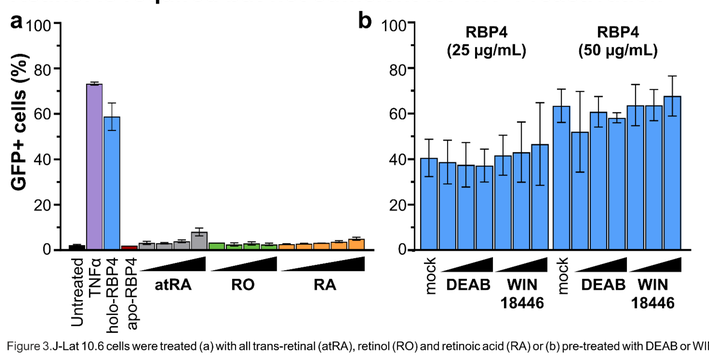


Figure 3.J-Lat 10.6 cells were treated (a) with all trans-retinal (atRA), retinol (RO) and retinoic acid (RA) or (b) pre-treated with DEAB or WIN18446 inhibiting conversion of retinal to retinoic acid and subsequently treated with RBP4. The % of GFP+ cells were determined by flow cytometry.

RBP4 activates HIV-1 through JAK/STAT5 and JNK signaling

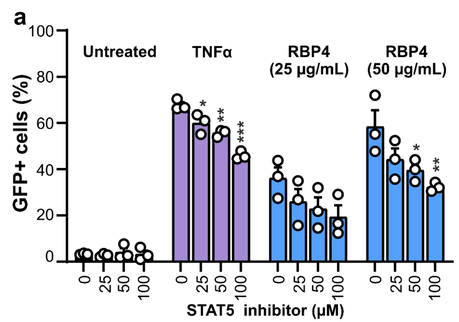
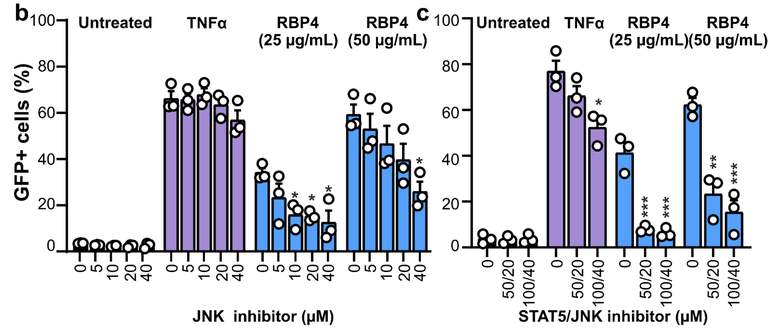


Figure 4. J-Lat 10.6 cells were pretreated with STAT5 (a) or JNK (b) inhibitor or their combination (c). 4 hours later, the cells were left untreated or treated with TNFα or RBP4. The percentage of cells showing HIV-1 eGFP reporter provirusewere determined by flow cytometry.



RBP4 reactivates latently infected cells from PLWH under ART

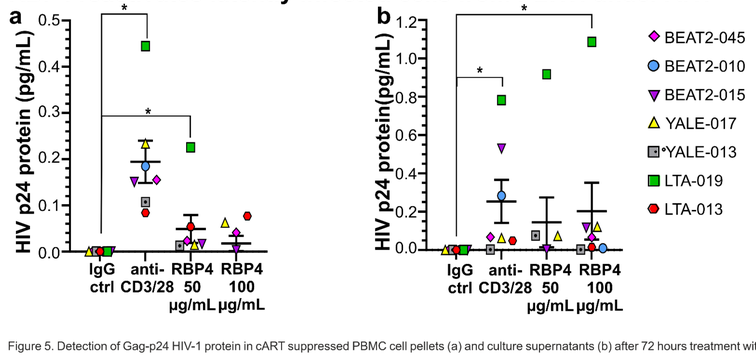


Figure 5. Detection of Gag-p24 HIV-1 protein in cART suppressed PBMC cell pellets (a) and culture supernatants (b) after 72 hours treatment with LRAs as measured by SIMOA.

CONCLUSIONS

Physiological concentrations of holo-RBP4 reverse HIV-1 latency in a variety of T cell lines and in PBMCs from PLWH after long-term cART

Activation of latent HIV-1 by RBP4 depends on JAK/STAT5 and JNK signaling but not on retinoic acid metabolism

RBP4 offers new insights into endogenous factors with the potential to reactivate HIV-1 latent reservoirs *in vivo*

OUTLOOK

Targeting of the JAK/STAT5 and JNK signaling pathways to discover their role on HIV latency reversal

Discovery of other endogenous LRAs

RBP4 is also an adipocytokine: is the size of the latent HIV-1 reservoirs reduced in obese PLWH?