Involvement of the mTOR Signaling Pathway in the Regulation of Antiretroviral Drug Efflux Transporters in CD4+ T-cells Exposed to an HIV Pseudotype

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BACKGROUND

- ATP-binding cassette (ABC) drug efflux transporters could contribute to low antiretroviral drug (ARV) intracellular concentrations in HIV-1 target tissues and cells.[1]
- Furthermore, studies have reported that the expression and function of these transporters could be induced in activated and/or HIV-infected T-cells.[2]
- The mammalian target of rapamycin (mTOR) signaling pathway is activated following HIV-1 infection and T-cell activation.[3]
- Therefore, we examined the regulation of ABC drug efflux transporters by mTOR, and their potential contribution to the inflammatory response following exposure of T-cells to an HIV pseudotype ($pHIV_{NL4-3}$).

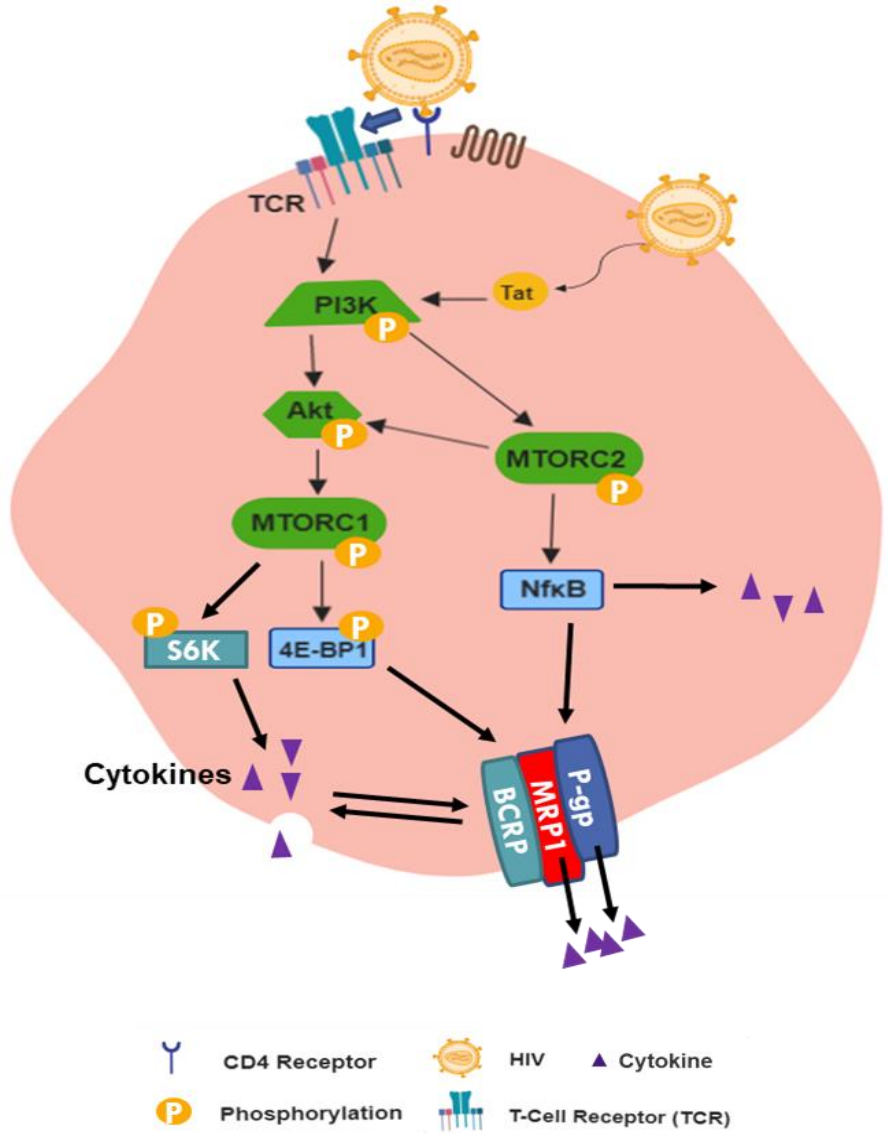


Figure 1. Potential involvement of mTOR in the regulation of drug efflux transporters and inflammatory response in HIV-infected T-cells.

1. Whyte-Allman and Bendayan (2020). HIV-1 Sanctuary Sites—the Role of Membrane-Associated Drug Transporters and Drug Metabolic Enzymes. AAPS J, 22 (118).
2. Turriziani O et al., (2008). Expression Levels of MDR1 , MRP1 , MRP4 , and MRP5 in Peripheral Blood Mononuclear Cells From HIV Infected Patients Failing Antiretroviral Therapy. J Med Virol, 771 (766-771).
3. Kumar B et al., (2017). Hyperactivation of mammalian target of rapamycin complex 1 by HIV-1 is necessary for virion production and latent viral reactivation. FASEB J, 31(180-191).

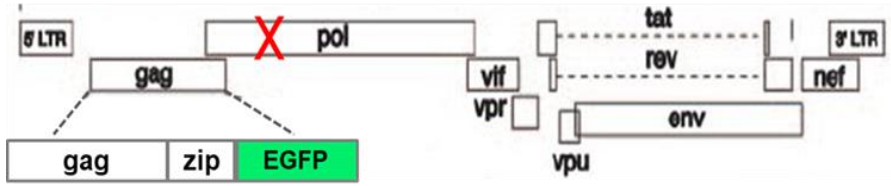
HYPOTHESIS

ABC transporters are upregulated in activated T-cells through the mTOR-mediated signaling pathway following exposure to $pHIV_{NL4-3}$.

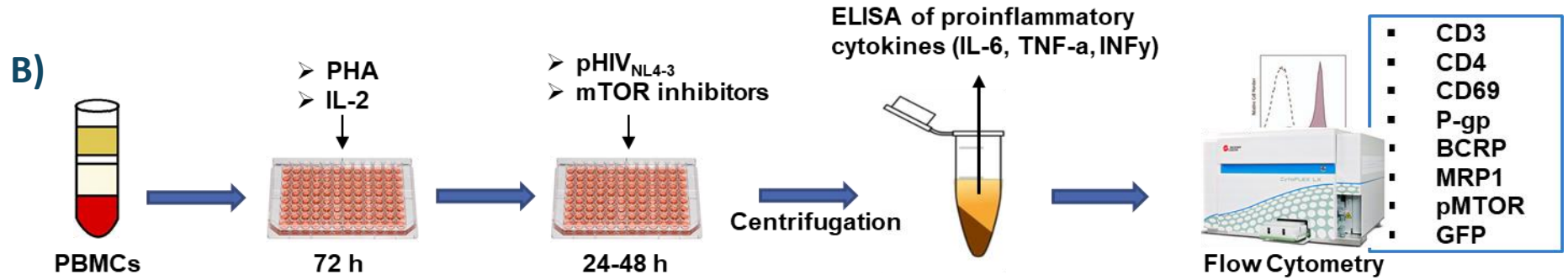
METHODS

A) HIV_{NL4-3} –VSVG Pseudotype ($pHIV_{NL4-3}$)

- Provided by Dr. Alan Cochrane, Dept. Molecular Genetics, UofT
- Deletion in regions of pol gene prevents viral replication.
- Modified with a GagzipGFP fusion to express EGFP.
- Pseudotyped with VSV envelope glycoprotein (VSVG).



B)



EGFP- Enhanced green fluorescent protein; ***PHA-*** Phytohemagglutinin A; ***VSVG***- Vesicular stomatitis virus glycoprotein

mTOR Inhibitor OSI-027 Reverses pHIV$_{NL4-3}$ Inductive Effects on Transporters and T-cell Activation

✓ **pHIV$_{NL4-3}$ increases the expression of ABC transporters and T-cell activation marker CD69 in CD4+ T-cells; this involves regulation by the mTOR signaling pathway.**

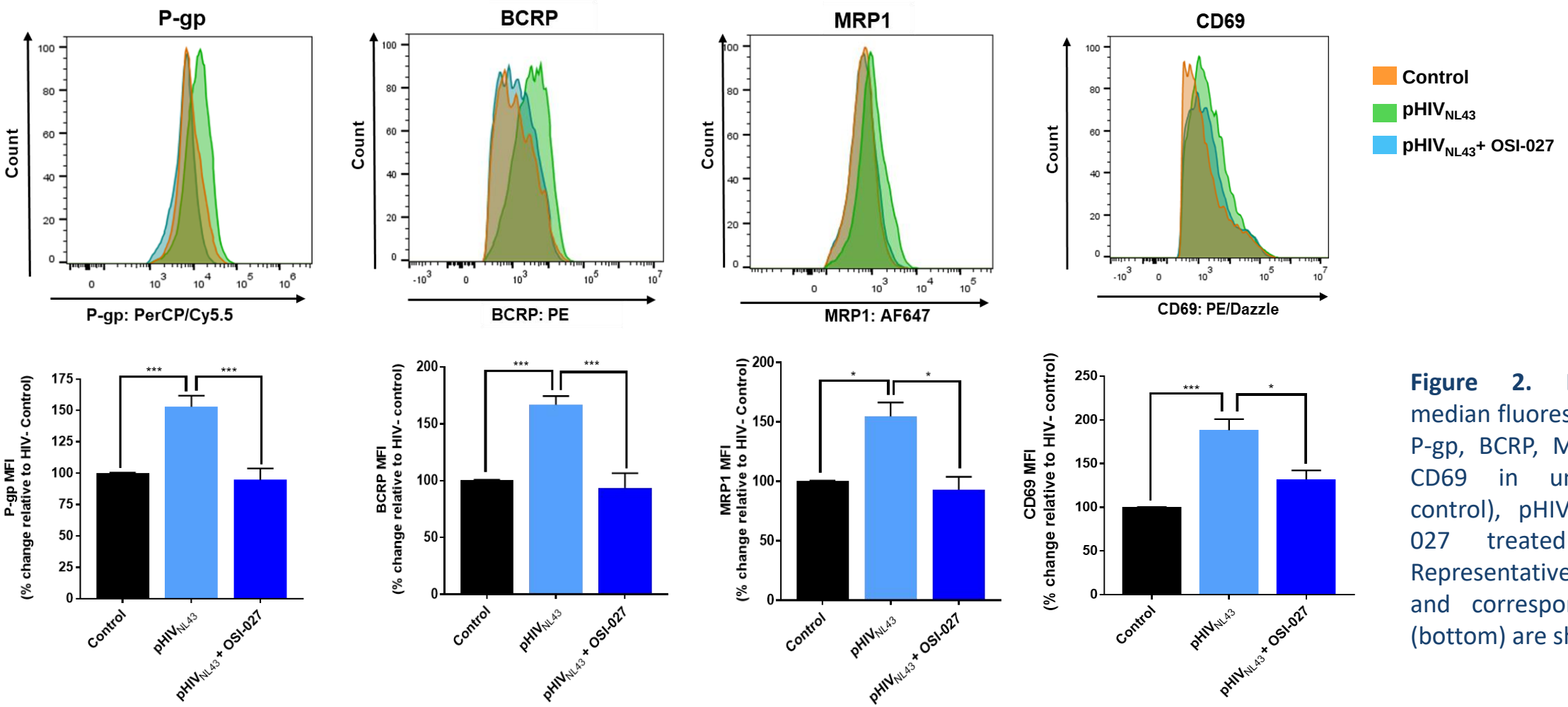


Figure 2. Expression (MFI; median fluorescence intensity) of P-gp, BCRP, MRP1, pmTOR and CD69 in untreated (vehicle control), pHIV$_{NL4-3}$, and/or OSI-027 treated CD4+ T-cells. Representative histograms (top) and corresponding bar charts (bottom) are shown.

Mean ± SEM; n = 3-4 donors per group; One-way ANOVA with Bonferroni's multiple comparisons test; *, p<0.05; ***, p<0.001.

mTOR, P-gp and MRP1 are Involved in Proinflammatory Cytokine Release from pHIV$_{NL4-3}$ Exposed PBMCs.

✓ Treatment with mTOR inhibitor OSI-027 decreased cytokine levels in supernatants of pHIV$_{NL4-3}$ exposed PBMCs.

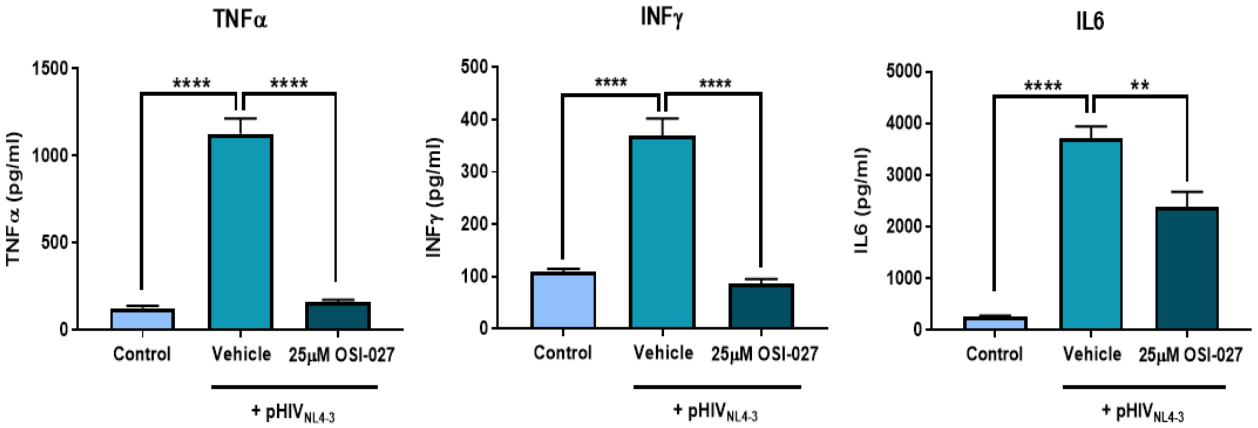


Figure 3. Proinflammatory cytokine concentrations in PBMC supernatants collected following exposure to vehicle, pHIV$_{NL4-3}$, and/or OSI-027 for 48h.

✓ Pharmacological inhibition of P-gp or MRP1 decreased cytokine levels in supernatants of pHIV$_{NL4-3}$ exposed PBMCs.

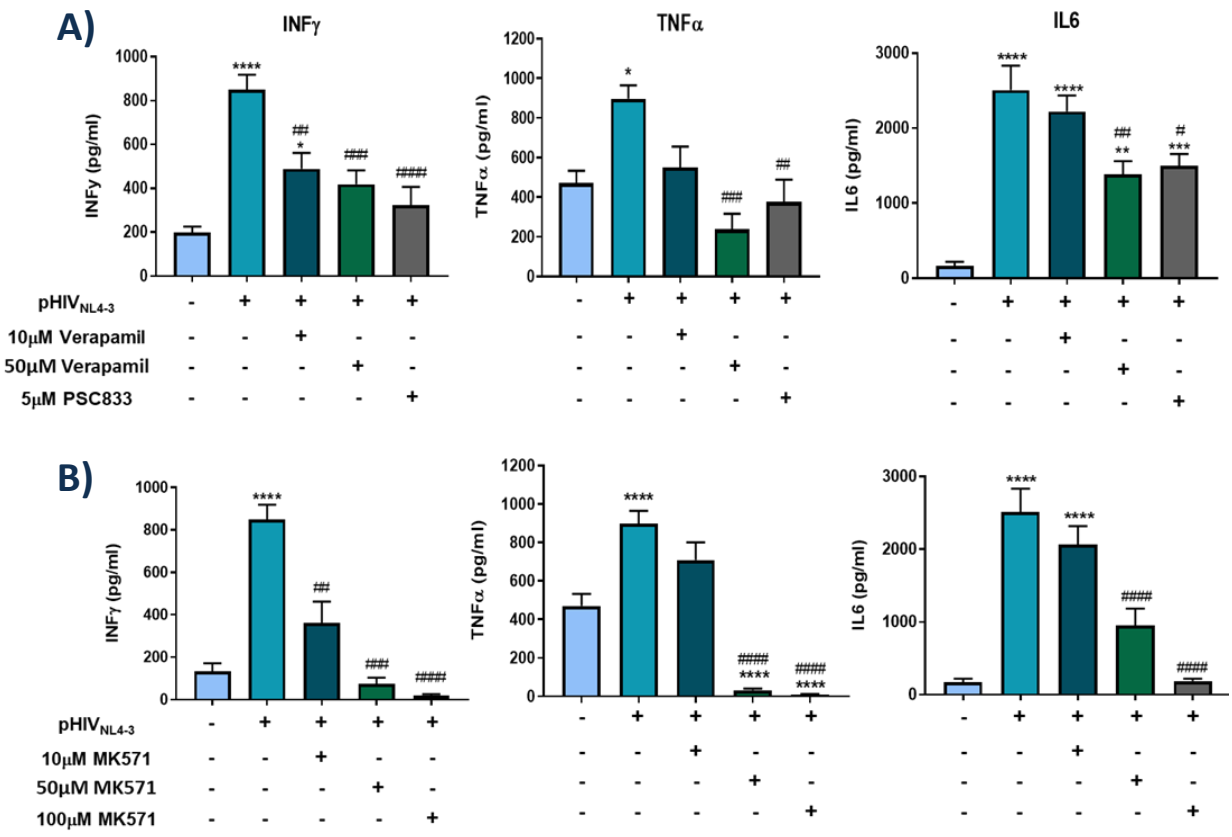


Figure 4. Proinflammatory cytokine concentrations in PBMC supernatants collected following exposure to vehicle, pHIV$_{NL4-3}$, and/or P-gp inhibitors verapamil and PSC833 **(A)** or MRP1 inhibitor MK571 **(B)** for 48h.

CONCLUSION

We present novel data demonstrating that ABC drug efflux transporters are upregulated via mTOR signaling in CD4+ T-cells exposed to pHIV$_{NL4-3}$. These transporters could limit ARV permeability in HIV target T-cells, as well as potentially contribute to HIV-associated proinflammatory cytokine secretion. This study provides a basis to further assess the role and regulation of ARV drug efflux transporters in T-cell activation and inflammatory response, in the context of HIV infection.

Mean ± SEM; n = 3 donors per group; One-way ANOVA and Bonferroni's post-hoc analysis; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001; #, p<0.05; ##, p<0.01; ###, p<0.001; ####, p<0.0001.