# IL-10 signaling contributes to SIV persistence in ART-treated rhesus macaques

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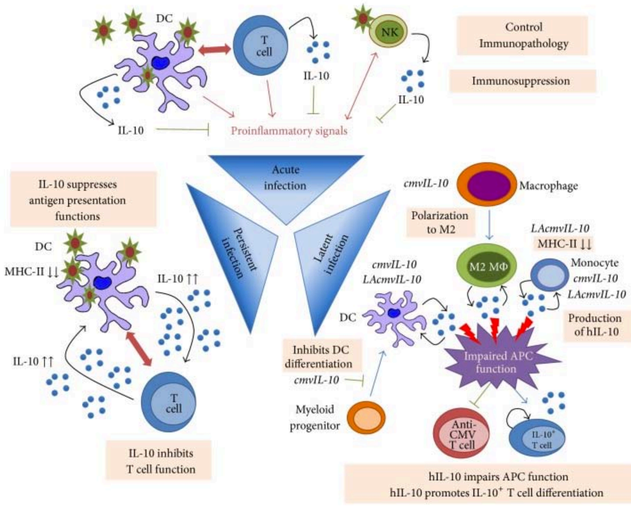
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Abstract # 376

## INTRODUCTION

A major obstacle to curing HIV infection includes the presence of a pool of long-lived, latently-infected CD4 T-cells, and infected cells actively transcribing vRNA within lymphoid tissues that persist despite effective ART (1). The exact mechanisms regulating the establishment and maintenance of this HIV reservoir are unknown, thus impeding the design of selective strategies limiting HIV persistence. Recently, T follicular helper (Tfh; CXCR5$^+$ PD-1$^{hi}$) CD4 T-cells in the B-cell follicle of secondary lymphoid organs have been characterized as major reservoir compartment under ART (2). Additionally, Tfh CD4 T-cells expand during chronic HIV infection (3) and SIV replication has been shown to be largely restricted to this cell population in elite controller RMs (4). Interleukin (IL-10) is an anti-inflammatory cytokine produced by several components of the adaptive and innate immune system (macrophages, dendritic cells, B-cells, and Tr1-cells). Mechanistically, IL-10 binds the IL-10R on dendritic cells and macrophages to reduce antigen presentation (5) and inhibits the production of Th1 cytokines, while promoting Th2 cytokine production, such as IL-4, IL-15, and IL-13 (6). Furthermore, some chronic viruses, such as Epstein-Barr virus encode IL-10 to promote an environment favoring persistence (7) and IL-10 deficiency in mice was found to prevent the persistence of pathogens (8).

Our ongoing work in human PBMCs shows that *in vitro* IL-10 stimulation enhances the survival of activated and infected T-cells including the Tfh cells. Additionally, IL-10 stimulation *in vitro* leads to an upregulation in the frequency of cells expressing PD-1, CTLA-4, and LAG-3 resulting in impaired effector responses. Therefore, we hypothesize that IL-10 plays a critical role in promoting HIV persistence *in vivo*.




The main immune regulatory functions exerted by IL-10, which favors viral persistence by polarizing a Th2 response coupled with diminished antigen presentation on APCs (9).

## AIMS

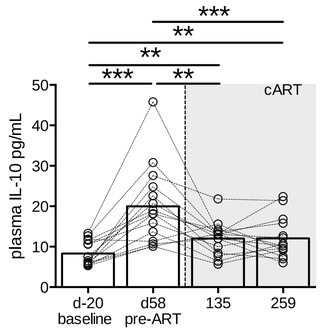
- Determine if plasma and lymph node levels of IL-10 are induced by SIV-infection and maintained at higher than physiological levels during ART treatment
- Correlate whether IL-10 levels associates with measures of reservoir establishment in viremic RMs.
- Identify whether IL-10 co-localizes with SIV-infected cells within the B-cell follicle
- Determine if plasma IL-10 is a predictor for the frequency of Tfh CD4 T-cell and SIV persistence under suppressive ART.

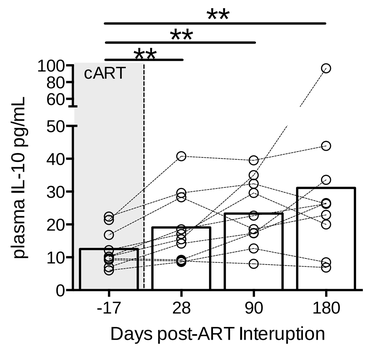
## RESEARCH DESIGN AND METHODS

15 *Mamu*-B*08$^-$ and –B*017$^-$ RMs were infected i.v. with 300 $TCID_{50}$ $SIVmac_{239}$. At day 60 p.i., all animals began a 5-drug ART regimen consisting of PMPA, FTC, raltegravir, and ritonavir-boosted darunavir. After 7 months of treatment (day 270 p.i.), all animals underwent an analytical ART interruption. The frequency and phenotype of lymphocytes were determined in PB, LN, and RB using flow cytometry. Plasma levels of IL-10 were determined using an ultrasensitive bead-based fluorescence assay on a Singulex Erenna Platform. PBMCs were subjected to RNASeq using an Illumina HiSeq 1000. IL-10/SIV-DNA were quantified in embedded LN using IHC and DNAscope. Statistics were conducted using non-parametric Spearman correlations and the Mann-Whitney test.

## RESULTS

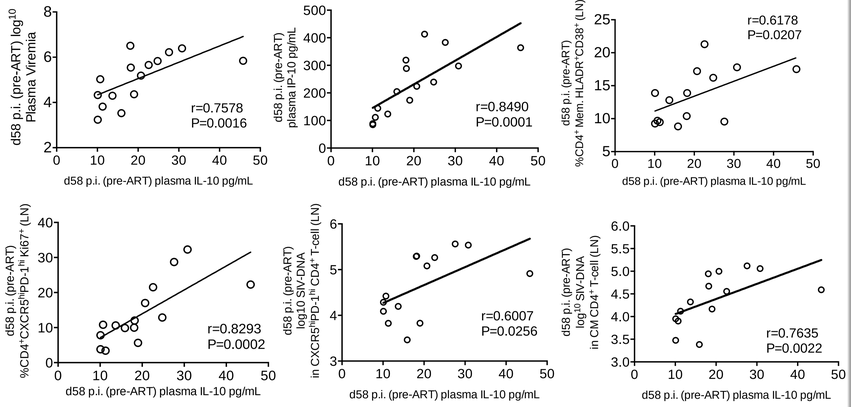
### Plasma IL-10 is elevated upon SIV infection and fails to normalize with ART



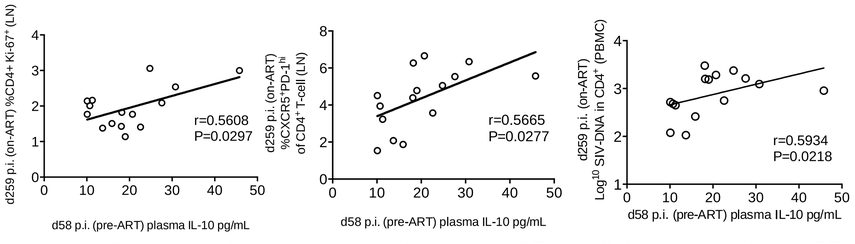

Plasma IL-10 levels were measured in RMs (n=15) at pre-infection (d-20), chronic infection (d58), and after 77 and 201 days of suppressive ART. Plasma IL-10 was then followed in 10 RMs following an analytical ART interruption. SIV infection results in (i) a significant increase in plasma IL-10, which (ii) fails to attenuate under long term ART; furthermore, (iii) plasma IL-10 levels rapidly rebound following the withdrawal of ART.

### In chronic infection, plasma IL-10 correlates with markers of disease progression and DNA content



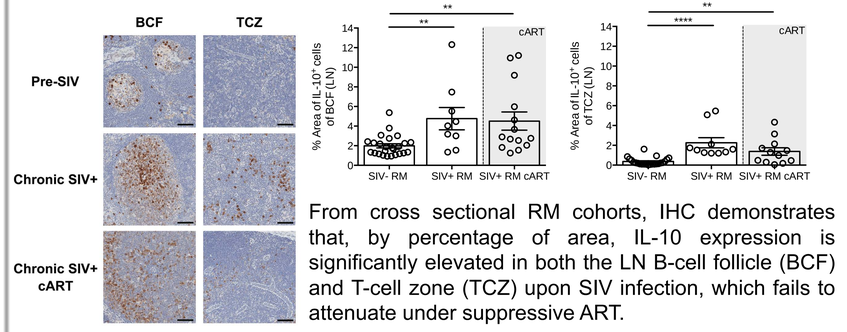

Plasma IL-10 at day 58 p.i. was correlated against known predictors of SIV disease progression in chronically infected RMs (n=15). IL-10 was found to correlate with (i) set point viremia; (ii) immune activation and inflammation as gauged by plasma IP-10 and HLA-DR$^+$CD38$^+$ CD4$^+$ and CD8$^+$ (data not shown) T-cells in the LN; (iii) proliferation and infection levels of the Tfh CD4+ T-cells in LN as gauged by Ki-67$^+$ and the SIV-DNA content; and (iv) the extent of infection in central memory CD4$^+$ T-cells in the LN

### Under suppressive ART, pre-ART plasma IL-10 predicts the frequency of Tfh CD4$^+$ T-cells and SIV-DNA$^+$ content in PBMCs



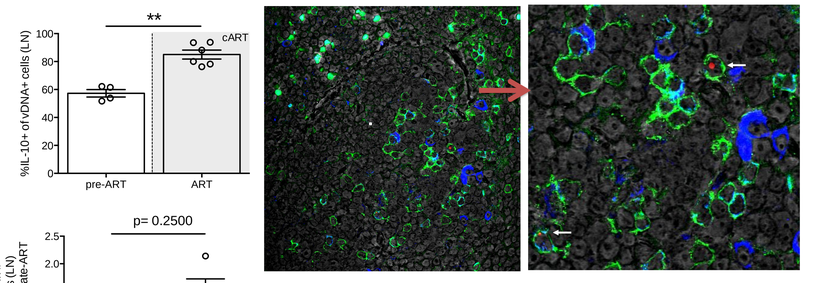

Markers of viral persistence were correlated under ART (day 258 p.i.) against pre-ART (day 58 p.i.) levels of plasma IL-10 in SIV-infected RMs (n=15). Pre-ART IL-10 was found to predict indirect measures of immune dysfunction and viral persistence such as (i) residual proliferation in LN-resident CD4$^+$ T-cells, (ii) the frequency of Tfh CD4$^+$ T-cells in the LN, as well as (iii) the reservoir size via SIV-DNA content in PBMCs and RB (data not shown).

### In ART-suppressed RMs, IL-10 identifies a majority of SIV-DNA$^+$ cells that persist in the LN B-cell follicle




From cross sectional RM cohorts, IHC demonstrates that, by percentage of area, IL-10 expression is significantly elevated in both the LN B-cell follicle (BCF) and T-cell zone (TCZ) upon SIV infection, which fails to attenuate under suppressive ART.

**vDNA – IL-10 – CTLA-4**




Using DNAscope in the LN BCF to co-stain for vDNA, IL-10, and CTLA-4, the majority of vDNA$^+$ cells co-localize with IL-10 in viremic RMs, which is further enriched under suppressive ART (up to 80%). Furthermore, as a longitudinal fold change (n=3), the number of IL-10$^+$ vDNA$^+$ cells remain more stable under ART relative to IL-10$^-$ vDNA$^+$ cells, suggesting enhanced maintenance mediated by IL-10 signaling.

## CONCLUSIONS

- Levels of IL-10 (plasma and LN), as well as IL-10 stimulated genes (data not shown) increase upon SIV infection and fail to normalize with ART.
- In chronic SIV infection, high IL-10 is associated with enhanced disease progression and immune dysfunction.
- Plasma IL-10 serves as a predictor for the size of the Tfh CD4$^+$ T-cell compartment and viral reservoir burden under suppressive ART.
- IL-10 co-localizes with majority of infected cells in the BCF, which remain markedly stable over time, suggesting preferential maintenance.

**IL-10 promotes and predicts SIV persistence during ART. Modulation of IL-10 represents a novel strategy towards an HIV cure.**

## ACKNOWLEDGEMENTS

We would like to thanks all the veterinary and animal care staff at Yerkes National Primate Research Center. Viral loads and cell-associated DNA were quantified by the Virology Core at Emory University, Jeffrey Lifson at NIH, and Nicolas Chomont at CRCHUM. Drugs for the ART regimens were provided by Drs. Geleziunas (Gilead Sciences), Kraus (Johnson & Johnson), and Hazuda (Merck). This work was supported by NIH grants AI116379, AI110334, AI122380, and AI104278 as well as by amfAR grant 109354-59-RGRL to M.P. This work was also supported in part with federal funds from the National Cancer Institute, National Institutes of Health (Contract No. HHSN261200800001E, JDL).

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