The Role of CD101 in HIV/SIV Pathogenesis and Maintenance of the Viral Reservoir

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INTRODUCTION

HIV remains a worldwide problem, with 38 million people living with this virus and a reported 1.8 million new infections and 1 million HIV-related deaths occurring each year. Understanding the early events after HIV infection is crucial for characterizing the underlying cause of viral pathogenesis resulting in immune dysregulation, chronic immune activation and viral persistence. While antiretroviral therapy (ART) has significantly reduced occurrences of HIV-related morbidities and mortalities, a therapeutic approach able to functionally cure HIV remains elusive. HIV-infected individuals must remain on lifelong ART due to persistence of latently infected cells which contain transcriptionally silent proviruses capable of evading immune surveillance.

CD101 is a type I transmembrane glycoprotein that has been linked to highly suppressive T_{Regs}[(1)] and was recently described to be selectively expressed on terminally differentiated and highly dysfunctional Ag-specific $CD8^+$ T cells during chronic LCMV infection and cancer[(2-4)]. Previously, we and other groups have shown that memory $CD4^+$ T cells expressing markers of exhaustion (PD-1, CTLA-4, LAG-3 and TIGIT) are enriched for viral DNA[(5-8)]. Here we track the longitudinal kinetics and phenotype of $CD4^+$ T cells expressing CD101 to determine their role in SIV/HIV infection.

AIMS

- ❖Immunologically characterize $CD101^+$ $CD4^+$ T cells during the course of SIV infection
  - ❖Phenotype and kinetics of $CD4^+$ T cell subsets expressing CD101
  - ❖Transcriptomic Signature of $CD101^{+/-}$ T_{Regs}
  - ❖Expression of co-inhibitory receptors
  - ❖Proliferation history
- ❖Identify whether $CD101^+$ $CD4^+$ T cells play a vital role in viral persistence

CONCLUSIONS

- ❖$CD101^+$ $CD4^+$ T cells are preferentially depleted during acute SIV infection and reconstitute during ART to baseline levels in all $CD4^+$ T cell subsets
- ❖$CD101^+$ $CD4^+$ T_{Regs} are transcriptionally distinct, more differentiated, and CD101 demarcates suppressive T_{Regs}
- ❖$CD101^+$ $CD4^+$ T cells are enriched for the exhaustion markers PD-1 and CTLA-4, and have elevated levels of cell cycling (Ki-67)
- ❖Based on results from the LARA model of latency induction, $CD101^+$ $CD4^+$ T cells preferentially enter latency and quiescence

$CD101^+$ $CD4^+$ T cells could be critical contributors to HIV pathogenesis and persistence and targets for future immunotherapeutic interventions.

RESEARCH DESIGN AND METHODS

28 *Mamu*-B*08$^-$ and B*017$^-$ RMs were infected intravenously with 300 $TCID_{50}$ $SIVmac_{239}$. A second group of RMs at day 42 post-infection (p.i.) began a 3-drug ART regimen (emtricitabine, FTC; tenofovir, TDF; and dolutegravir, DTG) that was maintained for at least 12 months. The frequency and phenotype of $CD101^+$ T cells were determined in blood, lymph node, and gut mucosal mononuclear cells using flow cytometry. Quantitative real-time reverse transcription (RT)-PCR was performed to determine SIV plasma viral load. Blood mononuclear cells were sorted on a FACSAria II and subsequent scRNAseq was performed using SMART-Seq2 protocols. Bioinformatic analysis was done using R 3.6.2, DESeq2, SimpleSingleCell, and SCE. Latency and Reversion Assay (LARA) was used for latency induction[(9)].

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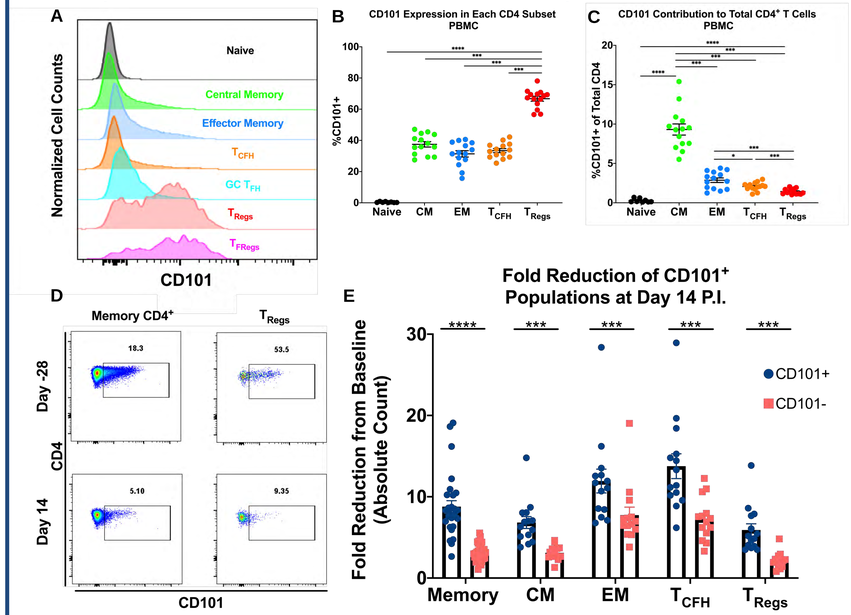
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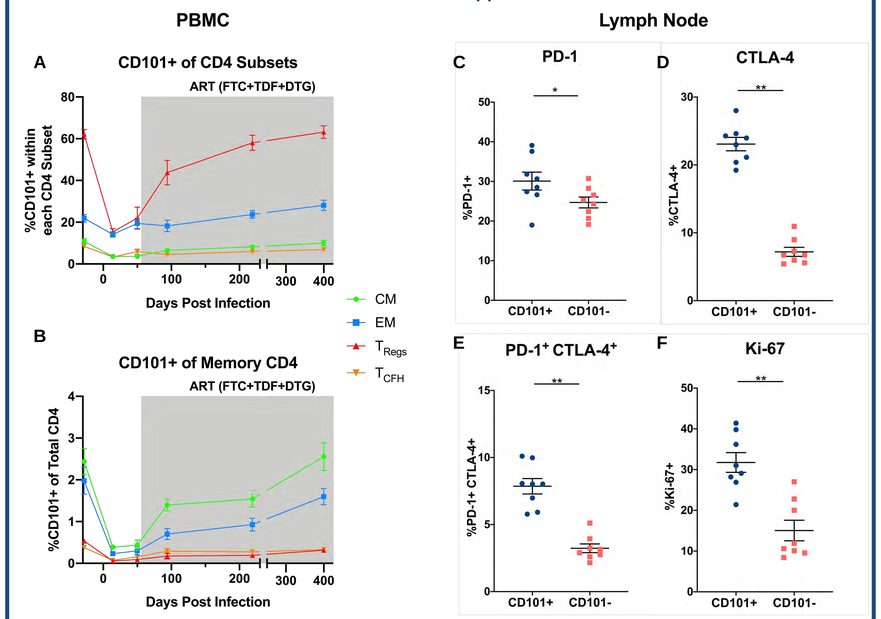
RESULTS

CD101 Expression on $CD4^+$ T Cell Subsets and Preferential Depletion During Acute SIV Infection



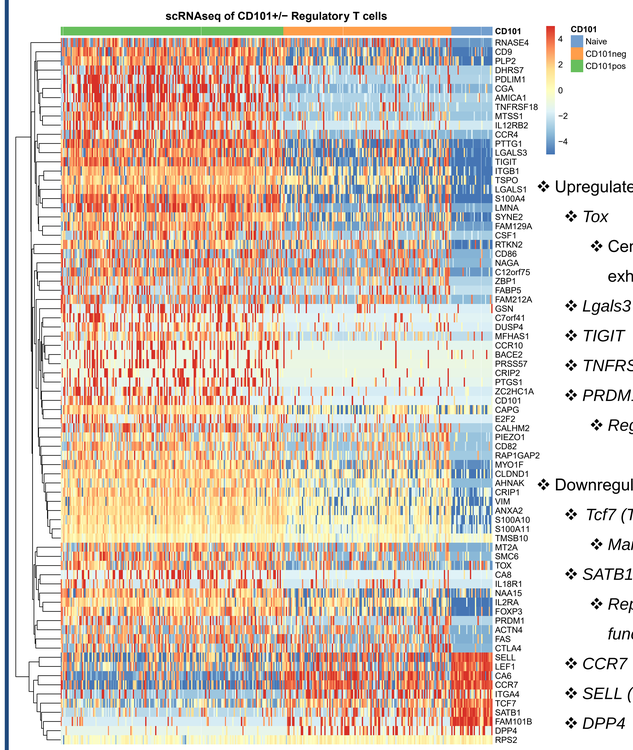
CD101 is highly expressed on T_{Reg} and T_{FReg} subsets and are depleted during acute infection. (A-C) Relative frequencies of CD101 expression within $CD4^+$ T cell in uninfected RMs. (Naïve: $CD28^+$ $CD95^-$ $CCR7^+$; CM: $CD95^+$ $CCR7^+$; EM: $CD95^+$ $CCR7^-$; T_{CFH} (Circulating T_{FH}): $CD95^+$ $CXCR5^+$ PD-1^+; and T_{Reg}: $CD95^+$ $CD25^+$ $CD127^-$ $FoxP3^+$) **(D)** Representative stain of CD101 expression in Memory CD4+ T cells and T_{Regs} at baseline and D14 p.i. **(E)** Depletion of CD101 $CD4^+$ T cells within each subset at Day 14 p.i. (n = 28 RMs). *p<0.05, ***, P < 0.001, ****, P < 0.0001.

CD101 Expression is Associated with Heightened Levels of Co-Inhibitory Receptors and Proliferation in ART Suppressed RMs



$CD101^+$ $CD4^+$ T cells return to baseline levels after >1 year of ART and this population of cells are enriched for Co-IRs and Ki-67. Longitudinal kinetics of $CD101^+$ $CD4^+$ T cell subsets during SIV infection and ART in PBMCs **(A and B)** (n = 8). Lines denote mean ± SEM. $CD101^+$ memory $CD4^+$ T cells within lymph nodes had elevated levels of co-expression with CTLA-4 and PD-1 **(C-E)**. Ki-67 levels were higher in memory $CD4^+$ T cells expressing CD101 within lymph nodes **(F)**. (n = 8) *p<0.05, **p<0.001

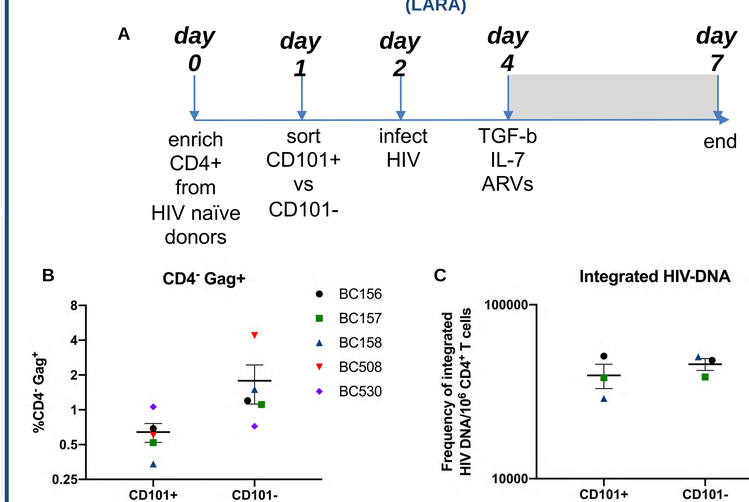
scRNAseq Analysis Shows that $CD101^+$ T_{Regs} are Transcriptionally Distinct and in a More Differentiated State



- ❖ Upregulated genes in $CD101^+$ T_{Regs}
  - ❖ *Tox*
    - ❖ Central regulator of T cell exhaustion
  - ❖ *Lgals3 (Galectin 3)*
  - ❖ *TIGIT*
  - ❖ *TNFRSF18 (GITR)*
  - ❖ *PRDM1 (BLIMP-1)*
    - ❖ *Regulates differentiation*
- ❖ Downregulated genes in $CD101^+$ T_{Regs}
  - ❖ *Tcf7 (TCF1)*
    - ❖ *Maintains stem-like features*
  - ❖ *SATB1*
    - ❖ *Repression results in suppressive function of* T_{Regs}
  - ❖ *CCR7*
  - ❖ *SELL (L-selectin)*
  - ❖ *DPP4*

scRNAseq of T_{Regs} indicates that $CD101^+$ T_{Regs} are transcriptionally distinct. Heatmap of top 50 differentially expressed genes between $CD101^+$ and $CD101^-$ T_{Regs} ($CD95^+$ $CD25^+$ $CD127^-$)

$CD101^+$ $CD4^+$ T Cells Trend to Viral Latency More Readily in the Latency and Reversion Assay (LARA)



$CD101^+$ $CD4^+$ T cells progress to a quiescent state more readily then $CD101^-$ $CD4^+$ T cells. (A) LARA Experimental layout. **(B)** Gag+ expression in sorted $CD101^+$ of $CD101^-$ populations at day 7. **(C)** Integrated HIV DNA measurements from sorted populations at day 7.