Dual therapy of Interleukin-21 and anti-α4β7 antibody administration during ART-treated SIV promotes immunological responses and ameliorates dysbiosis in rhesus macaques

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Abstract

Background: Despite effective antiretroviral therapies (ART), a cure for HIV remains elusive, necessitating new therapeutic strategies. Further, intestinal epithelium damage, mucosal immune depletion, and HIV-associated dysbiosis contribute to chronic immune activation and resultant sequelae, even during ART. Both IL-21 and anti-α4β7 administration modulate gut lymphocyte milieu improving mucosal barrier function, thus limiting inflammation and plasma viral loads (VLs). We hypothesized that combining these interventions would synergistically further improve outcomes.

Methods: Sixteen rhesus macaques (RMs) were inoculation with 300 TCID50 SIVmac239. Six weeks post acquisition (p.a.), ART (TDF+FTC+DTG) was initiated and administered until interruption at week 72 p.a. (ATI). Starting week 64 p.a., the experimental group (n=7; one excluded for remaining viremic during ART) was administered seven rounds of 100 μg/kg subcutaneous IL-21-IgFc weekly and 50 mg/kg intravenous anti-α4β7 antibody every three weeks. Animals were sacrificed at week 92 p.a. VLs, intact proviral DNA assays in CD4+ T-cells, flow cytometry, and fecal 16S rRNA sequencing were performed longitudinally.

Results: All RMs rebounded after ATI. Controls experienced progressive increases in VL reaching pre-ART setpoints after day 100 ATI. In contrast, and despite higher initial rebound, dual-treated RMs controlled viral replication better compared to pre-ART setpoints, with log10 2.2 copies/mL lower VLs by sacrifice (P<0.0001). At endpoint, dual-treated RMs had log10 1.4 copies/mL lower VLs than controls. Reservoir size was not different between groups. Following ATI, controls experienced increases in PD-1 expression on CD4+ TCMs (P<0.0001), with no significant changes in dual-treated RMs. Notably, reservoir size at dual therapy baseline correlated with PD-1+ TCMs (P=0.04) and predicted VLs ATI (P=0.007). Finally, dual therapy facilitated SIV-associated dysbiosis recovery (increased Firmicutes (P=0.001), decreased Spirochaetes (P=0.02), decreased Proteobacteria (P=0.02)) compared to controls (week 72 p.a.). *Roseburia* (a butyrate-producing Firmicute) abundance was predictive of PD-1+ TCMs after therapy (P=0.004) and subsequent viral loads (P=0.004).

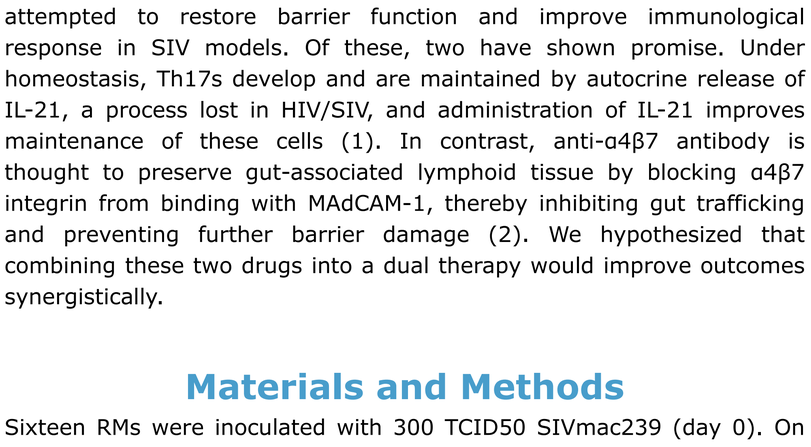
Conclusions: We demonstrate that combining IL-21 and anti-α4β7 treatments inhibits PD-1 expression on CD4+ TCMs, ameliorates dysbiosis, and limits VLs after ATI. These findings highlight the importance of targeting PD-1 and microbiome composition for improving immune responses against SIV and provide a roadmap for future mucosal immunotherapies in ongoing cure efforts.

Introduction

HIV is associated with significant gut pathology characterized by loss of epithelial integrity and depletion of Th17 cells. The resultant barrier dysfunction facilitates microbial translocation and chronic immune activation thereby causing accelerated biological aging and contributing to the elevated incidence of cardiovascular disease, metabolic dysregulation, and HIV-associated neurocognitive diseases (HAND) in people living with HIV (PLWH). Additionally, HIV/SIV-associated dysbiosis further increases these pro-inflammatory processes with a synchronous loss of commensal short-chain fatty acid-producing Firmicutes and increases in gram negative Bacteroidetes and pathogenic Spirochaetes and Proteobacteria. Several mucosal immunotherapies have been attempted to restore barrier function and improve immunological response in SIV models. Of these, two have shown promise. Under homeostasis, Th17s develop and are maintained by autocrine release of IL-21, a process lost in HIV/SIV, and administration of IL-21 improves maintenance of these cells (1). In contrast, anti-α4β7 antibody is thought to preserve gut-associated lymphoid tissue by blocking α4β7 integrin from binding with MAdCAM-1, thereby inhibiting gut trafficking and preventing further barrier damage (2). We hypothesized that combining these two drugs into a dual therapy would improve outcomes synergistically.

Materials and Methods

Sixteen RMs were inoculated with 300 TCID50 SIVmac239 (day 0). On day 56 p.a., daily cART (tenofovir disoproxil fumarate, emtricitabine, and dolutegravir) administration was initiated and continued until day 504. On day 448 p.a., dual therapy began for eight RMs, which included weekly IL-21-Fc administered subcutaneously for seven administrations (day 490 p.a.) and anti-α4β7 mAb infusions performed every three weeks until day 574 (day 70 ATI; seven administrations). At day 505 p.a., analytic cART interruption (ATI) began. Longitudinal plasma viral loads were determined from blood collected on days listed below. Intact proviral DNA assays (IPDA) was performed on CD4+ PBMCs to determine viral reservoir. Flow cytometry was utilized to characterize peripheral lymphocytes, with PD-1 expression on memory cells presented here. 16S rRNA sequencing was also performed longitudinally to characterize the bacterial microbiome which has been implicated in mucosal immunotherapy efficacy and several immune markers in HIV/SIV.



Results

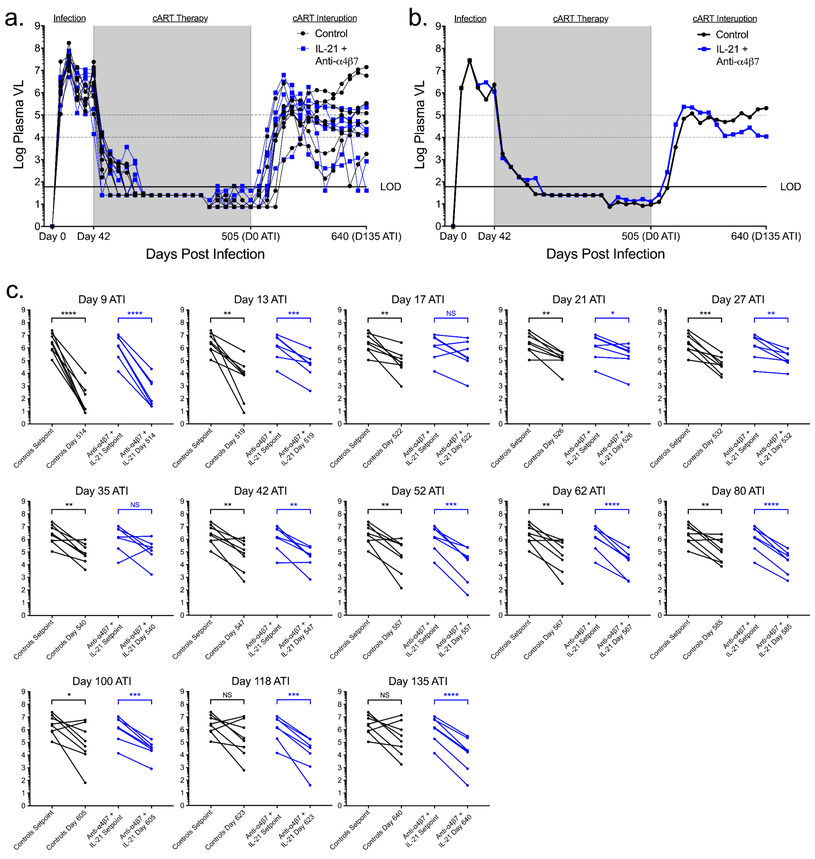
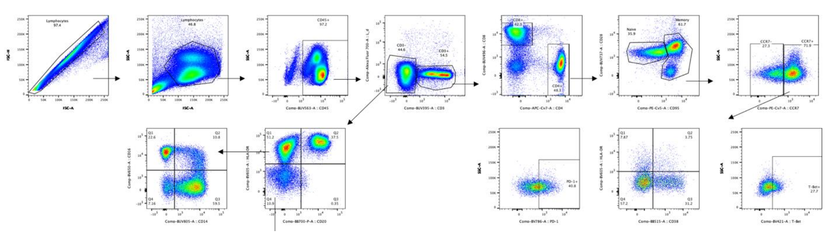


Figure 1: a) Longitudinal plasma VLs were monitored and reported in copies/mL. b) Geometric means of VLs. Difference at Day 135 ATI was log10 1.4 copies/mL lower following therapy compared with controls. c) Longitudinal comparison to setpoint. By Day 135 ATI, VLs were log10 3.4 lower in the dual-treated group compared with setpoint (* P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001)



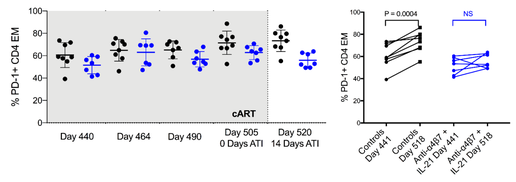


Figure 2: CD4+ Memory cells were gated into CCR7+ central memory and CCR7- effector memory phenotypes. PD-1 expression was determined on each subset. Therapy was associated with maintenance of lower PD-1 expression on each following ATI.

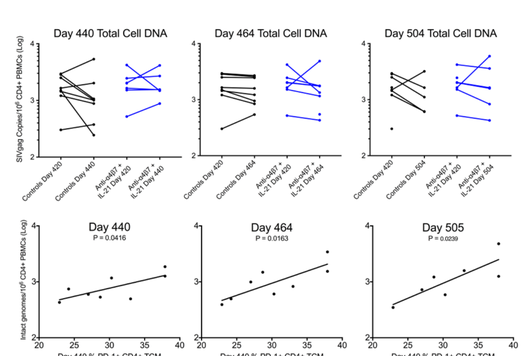


Figure 3: PD-1 expression on CD4+ TCMs prior to dual therapy predicts peripheral viral reservoir as determined by IPDA on CD4+ PBMCs.

Figure 4: Linear regression analysis was utilized to compare reservoir size before dual therapy initiation (Day 440) with rebound plasma VLs during ATI. Reservoir size was predictive of subsequent viremia. This association did not exist in controls RMs.

Results

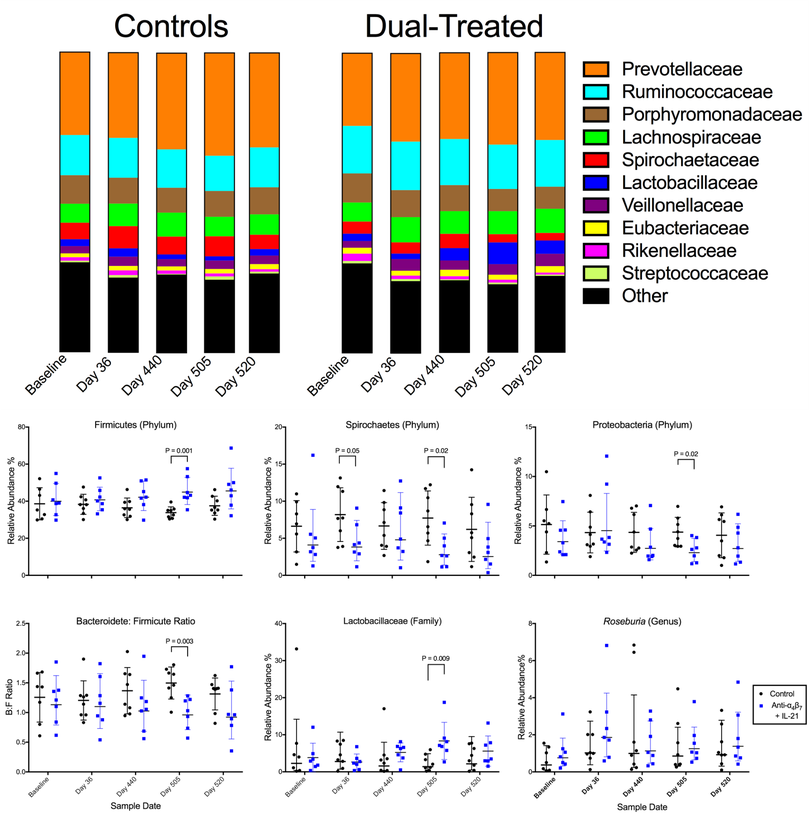


Figure 5: 16S sequencing was performed to determine the fecal bacterial microbiome longitudinal, including baseline, pre-cART (Day 36), therapy initiation (Day 440), final day of cART (Day 505), and two weeks ATI (Day 520). Family diversity is provided for comparison. Specific phyla that characterize HIV/SIV-associated dysbiosis are controlled (Spirochaetes and Proteobacteria), and the Bacteroidete: Firmicute Ratio is reduced in the dual-treated compared with controls.

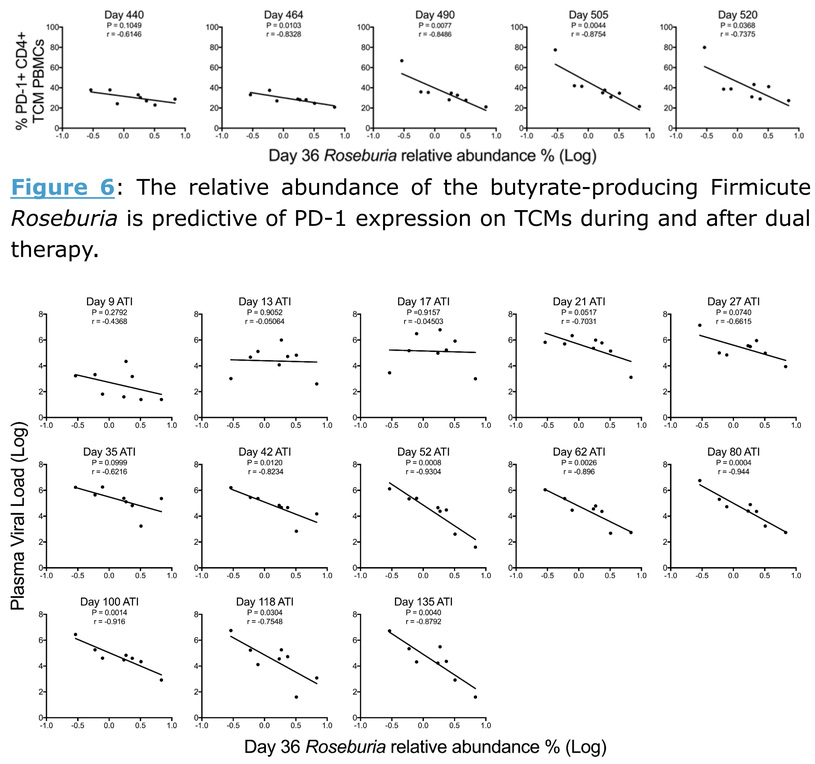
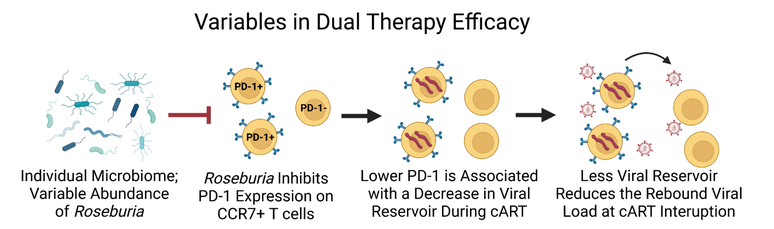


Figure 6: The relative abundance of the butyrate-producing Firmicute *Roseburia* is predictive of PD-1 expression on TCMs during and after dual therapy.

Figure 7: The relative abundance of *Roseburia* pre-cART is predictive of rebound plasma VL control in dual treatment.

Conclusions and Future Directions

Despite advances in cART, reservoir persistence and incomplete gut barrier healing remain as contributors to rebound viremia during cART disruptions and chronic immune activation, respectively. Herein, we show improvements in immune response and reductions in markers of HIV/SIV-associated dysbiosis. Further, two main factors seem to be predictive of improved viral control. These include baseline *Roseburia* abundance and PD-1 expression on TCMs, the cell type most enriched with provirus in PLWH. Previous studies have indicated that *Roseburia* may be critical for cancer immunotherapy, and *Roseburia* has been independently linked with immunological outcomes in PLWH and vedolizumab efficacy in inflammatory bowel diseases. Thus, the data presented herein suggests targeting either the microbiome or PD-1 with checkpoint inhibitors may offer benefit in future immunotherapy efforts.



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2. Byrareddy SN, Kallam B, Arthos J, Cicala C, Nawaz F, Hiatt J, et al. Targeting α4β7 integrin reduces mucosal transmission of simian immunodeficiency virus and protects gut-associated lymphoid tissue from infection. Nature Medicine. 2014;20(12):1397-400.

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