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Chronic Alcohol Increases CD8+ T-Cell Immunosenescence in Simian Immunodeficiency Virus-infected Rhesus Macaques

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Abstract

Background—Activated CD8+ T-cells correlate with viral load and may foretell antiretroviral therapy (ART) failure. HIV infection has been suggested to accelerate immunosenescence through chronic persistent inflammation. Alcohol-use disorders (AUD) are prevalent in persons living with HIV/AIDS (PLWHA). We tested the hypothesis that hazardous alcohol consumption accelerates immune activation and immunosenescence.

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Methods—Immune activation and immunosenescence were examined in CD8+ T lymphocytes (CD3+CD4–CD8+) isolated from intestinal biopsies, axillary lymph nodes, and peripheral blood mononuclear cells (PBMCs) of chronic binge alcohol (CBA)-consuming simian immunodeficiency virus (SIV)-infected male rhesus macaques with and without antiretroviral therapy (ART; CBA/ART+, CBA/ART–) and in PBMCs isolated from a cohort of PLWHA. Polychromatic flow cytometry was used to phenotype cells isolated from intestinal biopsies, lymph nodes, and peripheral blood from rhesus macaques and PLWHA. The Alcohol Use Disorders Identification Test (AUDIT) identified hazardous alcohol drinking in PLWHA. Viral load was determined by RT-qPCR and telomere length was measured using qPCR.

Results—PBMC CD8+ T-cell activation (CD38+HLA-DR+) and immunosenescence (CD28–) were increased over baseline levels ($857\% \pm 334$, $p < 0.05$; $398\% \pm 80$, $p < 0.05$, respectively) only in CBA animals not receiving ART. Viral load correlated with CD8+ T-cell immunosenescence in macaque PBMCs ($r_s = 0.49$, $p = 0.02$). Activated immunosenescent T-cell (CD8+CD38+CD28–) frequencies in PBMCs from PLWHA significantly correlated with AUDIT scores ($r_s = 0.75$, $p = 0.001$), while no correlation was observed with CD4+ T-cell and AUDIT scores ($r_s = -0.24$, $p = 0.38$). Activated immunosenescent T-cells had shorter telomeres than CD8+ T-cells (CD8+CD28+) from PLWHA.

Conclusions—Our results suggest that CBA and AUD augment immune activation and immunosenescence in SIV-infected macaques and PLWHA.

Keywords

alcohol; SIV; immune activation; immunosenescence; anti-retroviral therapy

Introduction

Immunosenescence is an expected phenomenon during aging by which both the innate and adaptive immune systems lose the ability to defend against infection or develop long-term immune memory after infection or vaccination (Franceschi et al., 2006; Hakim & Gress, 2007). Persistent immune activation driven by chronic antigenic burden leads to replicative exhaustion and senescence (Chou & Effros, 2013; Desai & Landay, 2010; Fülöp, Larbi, & Pawelec, 2013). With the availability of effective antiretroviral therapy (ART), human immunodeficiency virus (HIV) has transitioned into a chronic disease, and people living with HIV/AIDS (PLWHA) prematurely develop many geriatric medical conditions (Centers for Disease Control and Prevention; Deeks, Lewin, & Havlir, 2013; Harrison, Song, & Zhang, 2010). Furthermore, the immunopathy associated with HIV infection parallels aging-related immunosenescence (Deeks, 2011; Dock & Effros, 2011).

Alcohol-use disorders (AUD) are prevalent in PLWHA, with up to 50% reporting past or current AUD (Galvan et al., 2002). Independently, both HIV infection and alcohol abuse activate immune cells by promoting chronic persistent inflammation and an immunosuppressive state (Deeks, 2011; Keshavarzian, Fields, Vaeth, & Holmes, 1994; Keshavarzian et al., 1999; Molina, Happel, Zhang, Kolls, & Nelson, 2010; Rajasuriar et al., 2013; Zhang, Bagby, Happel, Raasch, & Nelson, 2008). The gastrointestinal tract is a particularly vulnerable target for both HIV and alcohol abuse. Early depletion of mucosal T-

cells in the intestine is a hallmark of HIV pathogenesis (Verhoeven, Sankaran, Silvey, & Dandekar, 2008).

We hypothesized that hazardous alcohol consumption in PLWHA accelerates the development of immunosenescence. To test this prediction we performed phenotyping for immunosenescent and immune-activated PBMCs isolated from rhesus macaques. We employed a rhesus macaque model of chronic binge alcohol (CBA) administration and simian immunodeficiency virus (SIV) infection with and without ART. SIV is an excellent model for studying HIV disease pathogenesis due to similarities in target cell tropism and disease course. We performed complementary phenotyping for immunosenescent PBMCs isolated from PLWHA with a spectrum of AUD. Finally, we measured telomere length in these PBMC subpopulations.

Materials and methods

Animals and experimental protocol

All experimental procedures were approved by Institutional Animal Care and Use Committees at Tulane National Primate Research Center (TNPRC) in Covington, LA and LSU Health Sciences Center in New Orleans, LA. This study was conducted on male rhesus monkeys (*Macaca mulatta*) of Indian origin that were 4–6 years of age. Data are compiled from a total of 21 animals. Other data collected from this cohort of animals, focusing on the impact of ART on the temporal viral load response in alcohol- compared to sucrose-treated animals was recently published (Molina et al., 2014). This report showed a sustained decrease in plasma viral load by ART and was not different between sucrose- and alcohol-administered animals. Animals were age- and body weight-matched, and randomized into four groups, all which were infected with SIV: 1) sucrose-administered without ART treatment (SUC/ART–); 2) sucrose with ART treatment (SUC/ART+); 3) CBA-administered without ART treatment (CBA/ART–); and 4) CBA-administered with ART treatment (CBA/ART+). Animals were individually housed in a Biosafety Level-2 building, and provided *ad libitum* water and standard primate chow (Lab Fiber Plus Primate Diet-DT, PMI Nutrition International, St. Louis, MO), supplemented with fruits. Alcohol (2.5 g/kg; ethanol 30% w/v water) or isocaloric sucrose administration began 3 months prior to SIV inoculation and continued throughout the study. Alcohol or sucrose was delivered daily via a permanently indwelling intragastric catheter (17 gauge; Access Technologies, Skokie, IL) that was attached to a cage-mounted swivel via a tether (Lomir Biomedical, Malone, NY), as previously described (Bagby, Zhang, Purcell, Didier, & Nelson, 2006). This protocol of alcohol administration achieved plasma alcohol concentrations between 50 and 60 mM. Macaques were inoculated intrarectally with 250 TCID of SIV_{mac251} provided by Dr. Preston Marx at the TNPRC, Covington, LA. SIV_{mac251} inoculum used in these studies is a virulent strain of SIV that is composed of multiple genotypes. Following infection of rhesus macaques with this isolate, high viral loads are observed and animals progress to end-stage disease typically within one year (Hatzioannou & Evans, 2012). Previous studies by our group have utilized aliquots of the same SIV_{mac251} stock (Nelson et al., 2013). SIV infection was determined by plasma viral load measurements 2 weeks post-inoculation.

Antiretroviral therapy

After 10 weeks of SIV infection, animals randomized to receive ART began treatment with two nucleoside reverse transcriptase inhibitors (NRTIs), tenofovir (PMPA, 9-R-2-[phosphonomethoxypropyl] adenine) and emtricitabine (FTC, beta-2, 3-dideoxy-3-thia-5-fluorocytidine) provided by Gilead Sciences, Foster City, CA. ART drugs were injected subcutaneously at a dose of 30 mg/kg/day each. The PMPA dose was decreased to 20 mg/kg after the initial 2 months of ART treatment. Non-ART treated animals were injected daily with an equal volume of vehicle. This ART protocol was selected based on published reports of NRTI efficacy in reducing viral load and at suppressing retrovirus replication by interfering with reverse transcriptase in rhesus macaques (Garcia-Lerma et al., 2008; Verhoeven et al., 2008). In addition, this drug combination has shown minimal toxicity in normal healthy rhesus macaques from infancy to adulthood, even after prolonged administration (Van Rompay et al., 2008).

Peripheral blood, lymph node, and intestinal mononuclear cell isolations

Peripheral blood samples, axillary and inguinal lymph nodes, and duodenal biopsies were obtained 1) at baseline (prior to any treatment), and 2) 12–14 months post-SIV inoculation during necropsy. Samples were collected from anesthetized animals, following an overnight fast. No alcohol was administered on the days samples were collected. Mononuclear cells (MC) from peripheral blood (PBMC), intestinal tissue, and lymph node samples were isolated and processed as previously described (Pahar et al., 2013). Briefly, PBMCs (human and rhesus) were enriched using a Ficoll (Invitrogen, Carlsbad, CA) density-gradient centrifugation. Intestinal and lymph node MCs were enriched using a Percoll (Invitrogen, Carlsbad, CA) gradient-density centrifugation. The tissue samples were resuspended in 37 °C complete Hanks Balanced Salt Solution (HBSS; Invitrogen, Carlsbad, CA) with 5% NCS and 5-mM EDTA and incubated in an orbital shaker for 30 min at 37 °C, followed by type II collagenase (60 U/mL; Sigma, St. Louis, MO) digestion in RPMI-5 for 1 h. Samples were then washed in RPMI 5 with aspiration through a 16-g needle, filtered through nylon mesh screen, and resuspended in RPMI-5. This was then layered on Percoll (Invitrogen, Carlsbad, CA) gradient according to manufacturer's instructions. Cells from the interface between 35% and 60% Percoll were collected, washed in PBS and resuspended in RPMI-5 for counting.

Flow cytometric analysis

MCs isolated from the peripheral blood, intestinal tissue, and lymph node were stained (2×10^6 , 0.5×10^6 , and 1×10^6 cells/sample, respectively) with a distinct antibody panel to determine T-cell populations. MCs were washed with RPMI-1640 + 10% FBS and resuspended in 100 μ L of PBS. The cells were stained for 30 min on ice with the following antibody panel: Am Cyan-Live/Dead, Qdot 655-CD8, APC-CD28 (Invitrogen, Grand Island, NY); Alexa Fluor 700-CD3, PE-Cy5.5-CD4 (BD Biosciences, San Jose, CA); FITC-CD38 (StemCell Technologies, Vancouver, BC, Canada); and ECD-HLA-DR (Beckman Coulter, Waltham, MA). Human PBMCs were stained with an antibody panel containing each of the following conjugated antibodies: V500-Live/Dead, BV650-CD8 (Invitrogen, Grand Island, NY); Alexa Fluor 700-CD3, APC-Cy7-CD4 (BD Biosciences, San Jose, CA); APC-CD28

(BioLegend, San Diego, CA); FITC-CD38 (StemCell Technologies, Vancouver, BC, Canada); PerCP-Cy5.5-HLA-DR (Beckman Coulter, Waltham, MA). The cells were washed with 2 mL of PBS, resuspended in 300 μ L of PBS + 1% paraformaldehyde (PFA) and acquired on an LSRII (BD Biosciences). Cytotoxic CD8⁺ T-cells were identified by CD3+CD4⁺CD8⁺ staining after gating on live singlets and forward and side scatter of the lymphocyte gate (CD3+CD4⁺CD8⁺; Supplementary Fig. A1 and A2). Data were analyzed using FlowJo (TreeStar, Ashland OR) software.

Plasma SIV viral load measurements

Plasma viral loads were measured by RT-qPCR as previously described with minor modifications (Bagby et al., 2003). Briefly, 500 μ L of plasma was centrifuged for 1 h at $16,000 \times g$ and 4 °C, and the pellets dissolved in 1 mL of Trizol reagent (Life Technologies, Grand Island, NY). RNA was extracted according to manufacturer's protocol and isolated RNA was resuspended in 25 μ L of RNase-free water. RNA concentration was measured using a NanoDrop (Thermo, Wilmington, DE). Reverse transcription was performed using the TaqMan Reverse Transcriptase Reagent Kit (Applied Biosystems, Grand Island, NY) for first strand synthesis, followed by qPCR with IQ Supermix (BioRad, Hercules, CA). Primers and probe to SIVgag were: forward GCG TCA TTT GGT GCA TTC AC, reverse TCC ACC ACT AGA TGT CTC TGC ACT AT, and FAM-labeled probe with Tamra quencher TGT TTT GCT TCC TCA GTA TGT TTC ACT TTC TCT TCT G. Quantification was determined by comparing sample Cp value to a standard curve of 10^1 to 10^7 gag copies, and calculated to copies/mL of plasma.

AUDIT assessments

The AUDIT is a self-report assessment consisting of 10 questions and is widely used as a screening tool to establish a patient's drinking behavior and to identify hazardous patterns of alcohol consumption (Donovan, Kivlahan, Doyle, Longabaugh, & Greenfield, 2006; Reinert & Allen, 2002). AUDIT questions address drinking frequency and quantity, binge drinking, impaired control over drinking, guilt related to drinking, and alcohol-related injuries (Saunders, Aasland, Babor, de la Fuente, & Grant, 1993). After informed consent, a random sample of HIV-infected subjects receiving care at the LSUHSC HIV Outpatient Clinic underwent AUDIT assessments and phlebotomy. This study was approved by the LSUHSC Institutional Review Board.

Telomere length assay

Telomere length was measured using the quantitative polymerase chain reaction (qPCR) method (Cawthon, 2002; Thomas, O'Callaghan, & Fenech, 2008). Genomic DNA was extracted using a QIAGEN DNeasy kit (Chatsworth, CA) per manufacturer's instructions, and concentration was measured using a NanoDrop (Thermo, Wilmington, DE). A standard curve for telomere length was generated by serial dilutions of known quantities of a synthesized 84bp oligonucleotide specifically containing TTAGGG repeats (14 repeats). The telomere standard curve was used to measure content of telomeric sequence per sample in kb. A second standard curve, for a single-copy gene (RPLP0, which encodes acidic ribosomal phosphoprotein P0), was generated by serial dilutions of known quantities of a

79bp oligomer corresponding to the amplicon. Each experimental DNA sample and standards were amplified using two primer pairs that target telomeric hexamer repeats and a single-copy gene (RPLP0). The telomere sequences and RPLP0 sequences were measured in separate PCR plates and all standards and samples are assayed in duplicate. The forward and reverse primer sequences for telomere amplification were: 5'CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT 3' and: 5'GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT 3', respectively. For RPLP0, the forward and reverse primer sequences were: 5'CAG CAA GTG GGA AGG TGT AAT CC 3' and 5'CCC ATT CTA TCATCA ACG GGT ACA A 3', respectively. For PCR amplification, reactions were incubated for 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, and 1 min at 60 °C.

Statistical analysis

Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical analyses of data were determined by nonparametric Mann-Whitney *t* test (for comparison between 2 groups) and two-way ANOVA (for comparisons among multiple groups). Pairwise comparisons were determined using Bonferroni *post hoc* test, and association was tested using Spearman's rank order correlation coefficient (r_s ; non-linear correlation) using GraphPad Prism 5 (GraphPad Software, Inc. La Jolla, CA). The sample size and specific test used for analysis are indicated in each figure legend. Statistical significance was defined as $p < 0.05$.

Results

CBA increases T-cell activation

CD8+ T-cell activation was defined by expression of both CD38 and HLA-DR. CD8+ T-cell activation in PBMC and intestinal MC fractions was significantly increased in CBA/ART– macaques relative to baseline levels ($857\% \pm 334$, $p < 0.05$ and $338\% \pm 83$, respectively, $p < 0.05$; Fig. 1A and 1B). No other treatment group's activation changes in PBMC or intestinal MC fractions reached statistical significance. Activated CD8+ T-cells in lymph nodes were not significantly increased over baseline in any of the treatment groups (Fig. 1C).

CBA increases T-cell immunosenescence

Utilizing the CD8+CD28– phenotypic definition for immunosenescence, we observed a significant increase in senescent CD8+ T-cells over baseline levels in PBMCs and lymph node MCs from CBA/ART– animals ($398\% \pm 80$, $p < 0.05$ and $285\% \pm 102$, respectively, $p < 0.05$; Fig. 2A and 2C). Moreover, there was a significant increase in PBMC in senescent CD8+ T-cells over baseline levels in CBA/ART– animals when compared to CBA/ART+ animals ($398\% \pm 80$ vs. $157\% \pm 52$, respectively, $p < 0.05$; Fig. 2A). Intestinal CD8+CD28– T-cells were not significantly altered in any of the treatment groups (Fig. 2B).

Immunosenescence correlates with viral load

Intrarectal SIV infection was confirmed using RT-qPCR to quantify plasma viral load 2 weeks post-inoculation. Viral load was serially measured throughout the course of the study and was significantly reduced in the ART+ animals (SUC/ART+, 2.7 ± 1.2 and CBA/ART+, 2.3 ± 0.9) at necropsy as compared with ART– animals (SUC/ART–, 5.4 ± 0.3 and CBA/ART–, 4.7 ± 0.6 , $p < 0.05$; Fig. 3A). This is in agreement with previous findings

obtained at earlier time points in the same cohort of animals (Molina et al., 2014). The frequency of immunosenescent CD8+CD28⁻ T-cells measured at necropsy and relative to baseline correlated ($r_s = 0.49$, $p = 0.02$; Fig. 3B) with viral load.

AUD correlates with activated immunosenescent CD8+ T-cells in PLWHA

To determine if our macaque data translates to humans, we analyzed human PBMCs isolated from PLWHA. AUDITs were performed on a random sample of 15 HIV-infected subjects representative of the HIV-infected population in New Orleans, including 6 women and 9 men, of which 11 were African-American and the remainder non-Hispanic whites (Table 2). The mean age was 55 ± 1 years (Table 2). The 6 subjects with an AUDIT ≥ 8 (potentially hazardous and harmful drinking threshold) were younger than the subjects with lower AUDIT scores (52 ± 2 vs. 57 ± 1 , $p = 0.03$, Table 2). The prevalence of immunosenescent CD8+ T-cells (CD8+CD28⁻) was not significantly correlated with AUDIT scores ($r_s = 0.29$, $p = 0.3$). In contrast, activated immunosenescent CD8+ T-cell (CD8+CD38+CD28⁻) frequency was significantly greater in PLWHA with higher AUDIT scores consistent with AUD and relative to PLWHA with low AUDIT scores (32 ± 10 vs. 7 ± 3 , $p < 0.05$; Fig. 4A). The frequency of activated senescent CD8+ T-cells positively correlated with AUDIT scores ($r_s = 0.75$, $p = 0.001$; Fig. 4B). Additionally, these activated immunosenescent T-cells have significantly shorter telomeres compared with CD8+CD28+ T-cells (941 ± 148 vs. 1664 ± 206 , $p < 0.05$; Fig. 4C). Plasma viral load did not differ among subjects with low AUDIT scores compared to high AUDIT scores (1.9 ± 0.4 vs. 3.6 ± 0.8 , $p = 0.12$, Table 2), and showed no correlation with activated immunosenescent T-cells ($r = 0.48$, $p = 0.22$, Supplementary Fig. A3), but was negatively correlated with percentage of CD4+ T-cells ($r_s = -0.72$, $p = 0.008$; data not shown).

Discussion

PLWHA exhibit increased risk for age-related comorbidities including immune dysfunction and, ultimately, immunosenescence (Hirschhorn, Kaaya, Garrity, Chopyak, & Fawzi, 2012; Papagno et al., 2004). We investigated the effects of CBA and SIV in a rhesus macaque model with and without ART administration. We predicted that HIV-induced immunosenescence is intensified in PLWHA by AUD. We found that CBA increased cytotoxic T-cell activation in the intestinal mucosa and in the peripheral blood in SIV-infected animals. CBA increased senescent cells in the blood and lymph nodes in animals infected with SIV. ART attenuated these perturbations. Additionally we found that PLWHA and AUD exhibited a greater proportion of activated, immunosenescent CD8+ T-cells in peripheral blood relative to PLWHA without AUD. Furthermore, we found that the activated, immunosenescent CD8+ T-cells had significantly shorter telomeres.

We examined CD8+ T-cell activation and immunosenescence as an indicator for systemic consequences of chronic inflammation resulting from CBA and SIV/HIV. We hypothesize that concurrent viral infection and CBA rather than either insult alone creates a greater chronic inflammatory milieu. We examined CD8+ T-cell activation and immunosenescence as an indicator for systemic consequences of chronic inflammation resulting from CBA and SIV/HIV. Our data indicate that CBA/SIV without ART increases CD8+ T-cell activation

and immunosenescence above baseline in circulating PBMCs and that there is a significant increase in immunosenescent CD8+ T-cells in CBA/ART- animals when compared to CBA/ART+ animals. SUC/ART- macaques did not show a similar increase. Within the intestinal compartment, CD8+ T-cell activation was significantly increased above baseline in CBA/ART- macaques while immunosenescence in CBA/ART- macaques was unchanged. In contrast, immunosenescence was significantly increased above baseline in lymph node CD8+ T-cells, while activation was unchanged. In PBMCs from CBA/SIV, ART treatment prevented the increase from baseline in the frequency of immunosenescent CD8+ T-cells. In addition, the frequency of activated immunosenescent CD8+ T-cells in the patient samples positively correlated with AUDIT scores, and these cells had significantly shorter telomeres, indicative of senescence. While these studies demonstrate increased immunosenescent CD8+ T-cells at this time point, we cannot determine whether these cells retain this phenotype over time. A limitation of measuring CD8+ cell activation with FACS is the large number of different activation markers that have been shown to be expressed on subsets of CD8+ T-cells (Caruso et al., 1997). We used the cell surface markers CD38 and HLA-DR to assess cytotoxic T-cell activation, since these markers have been used as a predictor of HIV disease progression (Giorgi et al., 1994; Kestens et al., 1992). We analyzed these activation markers on both non-senescent and senescent CD8+ T-cells. We differentiated these two subsets based on CD28 surface expression. Non-senescent T-cells have been shown to express CD28, which becomes down-regulated as the cell matures (Effros, 2004). Complete loss of CD28 has been widely used as an indication that CD8+ T-cells have become senescent. Human CD28 expression is ubiquitous at birth, and declines with age (Boucher et al., 1998; Effros, 2000). CD8+CD28- isolated from peripheral blood cells have been verified to lack the ability to re-enter the cell cycle (Effros et al., 1996). Another limitation of these studies is that they lack functional assays. Future studies are required to assess cell function of the CD8+ T-cell subpopulations.

Our results largely corroborate previous findings from other groups regarding immune activation, immunosenescence, and HIV disease (Desai & Landay, 2010; Fülöp et al., 2013). However, few studies have focused on the combined effects of alcohol and HIV infection with and without ART on immune system activation and immunosenescence. Due to the high percentage of PLWHA with AUD, our research is timely and emphasizes the importance of assessing patients for AUD. Although our human PBMC sample size was limited, the fact that we found significant correlations between activated immunosenescent CD8+ T-cells and AUDIT scores and that these activated immunosenescent CD8+ T-cells have shorter telomeres, suggests this phenomenon requires immediate attention. Furthermore, the similarity in patterns of immunosenescence observed in the CBA/SIV macaques to that seen in PLWHA with high AUDIT scores underscores the relevance of the animal model to the clinical setting. While this study identifies a role for alcohol-accelerated immunosenescence in SIV and HIV, further studies are critical to determine the mechanisms involved in alcohol-induced immunosenescence in the presence of SIV/HIV. Finally, we assert that HIV-associated immunosenescence may be partly due to health behaviors, such as alcohol abuse, rather than simply related to the virus.

Conclusions

CBA and AUDs exacerbate immune activation and immunosenescence in CD8+ T lymphocytes in SIV and HIV disease. These results suggest that AUD accelerates biological aging in PLWHA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Immune activation by chronic antigenic burden leads to CD8 T-cell immunosenescence.
- Alcohol use disorders (AUD) are prevalent in persons living with HIV/AIDS (PLWHA).
- Chronic binge alcohol increases immunosenescence in SIV-infected macaques.
- AUD increases immunosenescence in PLWHA, and these cells have shorter telomeres.
- These data emphasize the importance of assessing HIV+ patients for AUD.

Fig. 1.

CD8+ T-cell activation (CD3+CD4-CD8+CD38+HLA-DR+) expressed as a percent of baseline in (a) PBMCs, (b) intestinal MCs, and (c) lymph node MCs isolated from SIV-infected macaques. Two-way ANOVA was performed to determine statistically significant differences from baseline and Bonferroni *post hoc* to compare between groups. * indicates $p < 0.05$ from baseline values; $n = 5-6$ /group.

Fig. 2. CD8+ T and immunosenescence (CD3+CD4-CD8+CD28-) expressed as a percent of baseline in (a) PBMCs, (b) intestinal MCs, and (c) lymph node MCs isolated from SIV-infected macaques. Two-way ANOVA was performed to determine statistically significant differences from baseline and Bonferroni *post hoc* to compare between groups. * indicates $p < 0.05$ from baseline values, # indicates $p < 0.05$ compared to CBA/ART+; $n = 5-6$ /group.

Fig. 3.

(a) Viral load measured in plasma after 12–14 months of SIV infection from SIV-infected macaques. (b) Viral load correlated with CD8+ T-cell senescence in PBMCs from SIV-infected macaques. Two-way ANOVA with Bonferroni *post hoc* was performed to determine statistically significant differences between groups (a), and association was tested using Spearman's correlation (b). # indicates $p < 0.05$ compared to ART- groups; $n = 5\text{--}6/\text{group}$.

Fig. 4.

(a) Percentage of activated immunosenescent (CD3+CD4-CD8+CD38+CD28-) T-cell human PBMCs isolated from HIV-positive patients with either a low or high AUDIT score. (b) Correlation of AUDIT score and activated immunosenescent T-cells human PBMCs isolated from HIV-positive patients. (c) Telomere length of CD8+CD28+, CD8+CD38-CD28- and activated immunosenescent T-cells. Student's *t* test (a and c) was performed to determine statistically significant differences between groups, and association was tested using Spearman's correlation (b). * indicates $p < 0.05$ compared to AUDIT < 8 (a), * indicates $p < 0.05$ compared to CD8+CD28+ T-cells (c); $n = 6-9/\text{group}$.

Table 1

List of abbreviations

Abbreviation	Definition
ART	Antiretroviral therapy
AUD	Alcohol-use disorder
AUDIT	Alcohol use disorder identification test
CBA	Chronic binge alcohol
HIV	Human immunodeficiency virus
NRTI	Nucleoside reverse transcriptase inhibitors
PLWHA	People living with HIV/AIDS
SIV	Simian immunodeficiency virus
SUC	Sucrose

Table 2

Average Subject Demographics

Characteristic	Overall	AUDIT <8	AUDIT >8
Subjects	15	9	6
Mean age, y (SEM)	55 (1)	57 (2)	52 (1)*
Male (%)	60	67	60
Black (%)	73	67	83
Viral load ([Log] copies/mL, SEM)	2.6 (0.4) [#]	1.9 (0.4)	3.6 (0.8)

Demographics of patient sample used in the study.

* indicates $p < 0.05$ compared to AUDIT <8,

[#] Three patients did not have viral loads measured at the time of study enrollment. Specific details on each patient are outlined in Supplementary Table A1.