Published in final edited form as:

J Neurovirol. 2018 August ; 24(4): 420–431. doi:10.1007/s13365-018-0630-8.

Caspase-1-associated immune activation in an accelerated SIV-infected rhesus macaque model

Alison C. Kearns¹, Jake A. Robinson¹, Masoud Shekarabi¹, Fengming Liu¹, Xuebin Qin¹, and Tricia H. Burdo¹

¹Department of Neuroscience, Lewis Katz School of Medicine, Temple University, MERB 755, 3500 North Broad Street, Philadelphia, PA 19140, USA

Abstract

In the antiretroviral therapy (ART) era, chronic HIV infection is primarily associated with chronic inflammation driving comorbidities such as cardiovascular disease and neurocognitive impairment. Caspase-1 activation in leukocytes has been documented in HIV infection; however, whether caspase-1 activation and the downstream pro-inflammatory cytokines interleukin-1beta (IL-1 β) and interleukin-18 (IL-18) contribute to chronic inflammation in HIV comorbidities remains undetermined. The relationship between the caspase-1 cascade and persistent inflammation in HIV has not been investigated. Here, we used an accelerated simian immunodeficiency virus (SIV)-infected rhesus macaque model with or without ART to investigate the dynamics of caspase-1 and immune cell activation before infection, 21 days post infection (dpi), and necropsy. Caspase-1, IL-18, IL-1 β , and immune markers were measured both in the circulation and lymphoid tissues. We found a significant increase in caspase-1 and IL-18 in SIV infection that positively correlated with inflammatory monocytes and negatively correlated with CD4⁺ T cell counts. ART attenuated these effects at necropsy in the circulation. Further, lymph nodes from SIV+ or SIV+ART animals had increased activation of caspase-1 and potential upstream priming of the NF- κ B pathway, indicating that tissue-specific immune activation persists with ART. Together, these results shed light on the interconnectedness of the caspase-1 pathway and peripheral immune activation and further indicate that ART is not sufficient for suppressing inflammation. The caspase-1 pathway may provide novel therapeutic targets to improve HIV-associated comorbidities and health outcomes in the context of viral suppression.

Keywords

HIV; Caspase-1; Inflammation; HIV-associated comorbidities; SIV

Tricia H. Burdo burdot@temple.edu.

Author contributions Principal contributions of the authors are project conception/design (THB, XQ), data acquisition and analysis (AK, JAR, MS, FL), statistical analysis and interpretation (AK, JAR, XQ, THB), drafting of the manuscript (AK, JAR, XQ, THB), and critical revision of the manuscript (AK, JAR, XQ, MS, FL, THB).

Compliance with ethical standards

Conflicts of interests The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

Introduction

The course of human immunodeficiency virus (HIV) infection has changed dramatically since the introduction of combination antiretroviral therapy (ART). The life expectancy of patients living with HIV (PLWH) is approaching that of the general population and AIDS-related opportunistic infections are no longer the leading cause of death (Lohse et al. 2007; Kearns et al. 2017a). Chronic HIV infection is associated with an increased prevalence of comorbidities, including cardiovascular disease (CVD), neurocognitive impairment, metabolic syndromes, and malignancies (Effros et al. 2008; Alcaide et al. 2013). PLWH have up to a two-fold increased risk of CVD compared to age-matched uninfected individuals (Kearns et al. 2017a; Currier et al. 2003; Triant et al. 2007; Smith et al. 2014). The implementation of ART has resulted in a 40–50% decrease in HIV-associated dementia (HAD), the most severe form of HIV-associated neurocognitive disorder (HAND); however, the percentage of PLWH with HAND remains steady but with a more mild presentation (Saylor et al. 2016). Thus, ART is not sufficient for preventing or treating these comorbidities and a better understanding of residual immune activation with ART can aid in designing novel therapeutics.

The main targets of HIV and simian immunodeficiency virus (SIV) infection are T cells and monocytes/macrophages, resulting in T cell depletion and myeloid cell activation. Specifically, myeloid cells are important as both a reservoir for active viral replication and for viral persistence (Burdo et al. 2013; Porcheray et al. 2006), implicating this lineage as main players in HIV-associated comorbidities (Kearns et al. 2017a; Burdo et al. 2013; Hasegawa et al. 2009; Crowe et al. 2010). CD163, a macrophage-specific scavenger receptor, is shed during immune activation as soluble CD163 (sCD163). Plasma sCD163 levels correlate with non-calcified plaques in HIV-associated CVD, HIV-associated brain pathology, severity of HAND, and metabolic syndrome (Burdo et al. 2013; Royal 3rd et al. 2016; Subramanian et al. 2012; Bryant et al. 2017; Fourman et al. 2018; Fitch et al. 2013; Burdo et al. 2011a). Soluble CD14 (sCD14) is another monocyte/macrophage activation marker increased by inflammatory triggers such as LPS, interleukin-6 (IL-6), and interleukin-1beta (IL-1 β) (Shive et al. 2015). sCD14, like sCD163, is a predictor of both overall morbidity and mortality in HIV disease, as well as an indicator of CVD (Sandler et al. 2011; Tenorio et al. 2014; Kelesidis et al. 2012). In PLWH on ART, HIV infection not only activates immune cells but also triggers an array of molecular pathways, including nod-like receptor protein 3 (NLRP3) inflammasome-mediated caspase-1 activation (Kearns et al. 2017a; Chivero et al. 2017; Walsh et al. 2014; Hernandez et al. 2014; Guo et al. 2014). However, the mechanisms underlying HIV-immune cell activation require further investigation (Kearns et al. 2017b).

Increased caspase-1 activation, namely through inflammasome formation in immune cells, has been documented in different stages of HIV infection (Kearns et al. 2017a). Inflammasome formation is a highly conserved process of the innate immune system in response to pathogens, including HIV. Pattern recognition receptors identify infectious agents that trigger downstream mechanisms, including activation of inflammasomes. NLRP3 inflammasome formation, and subsequent caspase-1 activation leads to the release of active IL-1 β and IL-18, contributing to chronic inflammation (Kearns et al. 2017a; Guo et al. 2015).

With HIV infection, caspase-1 activation releases IL-1 β and IL-18 from monocytes/macrophages (Hernandez et al. 2014; Guo et al. 2014; Chattergoon et al. 2014). Caspase-1 is activated in microglia from animal models of HAND and cognitively impaired PLWH (Chivero et al. 2017; Walsh et al. 2014; Mamik et al. 2017). Similarly, the caspase-1 pathway, measured by IL-18, may be driving cardiovascular disease in SIV infection in animals on a high-fat diet (Yearley et al. 2009). Further, inflammasome formation and caspase-1-induced pyroptosis contributes to CD4⁺ T cell depletion in uncontrolled HIV infection (Doitsh et al. 2014). These results indicate a critical role of the caspase-1 pathway in the pathogenesis of HIV and SIV infection. However, the relationship of caspase-1 and peripheral immune cell activation during the course of HIV infection has not been investigated. This study provides a preliminary examination of the role of caspase-1 in driving persistent inflammation in the context of ART and contributing to the pathogenesis of HIV-associated comorbidities.

In this study, we measured active caspase-1 and its downstream cytokines IL-1 β and IL-18 in the plasma over the course of SIV infection, in the presence of ART, and in hematopoietic and lymphoid tissues at necropsy. We used a well-characterized nonhuman primate model of SIVmac251 infection with subsequent CD8 depletion to accelerate disease (Burdo et al. 2010; Walker et al. 2014; Williams and Burdo 2012; Lakritz et al. 2015a; Lakritz et al. 2015b), enabling the study of the relationship between viral replication, immune cell activation, and cytokine responses in a controlled experimental setting. Implementation of a contemporary ART regimen elucidated the relationship of caspase-1 activation to markers of monocyte/macrophage activation with successful viral suppression. Better understanding of persistent immune activation with ART is necessary for determining the underlying causes of comorbidities.

Materials and methods

Animals used in the study and ethical statement

Sixteen male, Indian rhesus macaques (*Macaca mulatta*) were used in this study (Table 1). Three rhesus macaques (A01-A03, SIV-) served as uninfected controls. Seven animals (A04-A10, SIV+) were inoculated intravenously with SIVmac251 viral swarm (5 ng p27; Tulane National Primate Research Center's (TNPRC; Covington, LA) Viral Core) and subsequently CD8-depleted through administration of 10 mg/kg of anti-CD8 antibody subcutaneously at 6 days post-infection (dpi) and 5 mg/kg of antibody intravenously at 8 and 12 dpi (Nonhuman Primate Reagent Resource). The SIV+ animals were sacrificed according to humane endpoints consistent with the recommendations of the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals. The development of simian AIDS was determined post-mortem by the presence of *Pneumocystis carinii*-associated interstitial pneumonia, *Mycobacterium avium*-associated granulomatous enteritis, hepatitis, lymphadenitis, and/or adenovirus infection of surface enterocytes in both small and large intestines. Six animals (A11-A16, SIV+ART) were not only SIV-infected and CD8 depleted, but also received a triple ART regimen of Raltegravir (22 mg/kg oral twice daily, Merck), Tenofovir (30 mg/kg subcutaneous once daily, Gilead), and Emtricitabine (10 mg/kg subcutaneous once daily, Gilead) at 21 dpi until the timed sacrificed at 118–120 dpi.

Animals were anesthetized with ketamine-HCL and euthanized by intravenous pentobarbital overdose. Animals used in the study were housed at the TNPRC. All animals used in this study were handled in strict accordance with American Association for Accreditation of Laboratory Animal Care with the approval of the Institutional Animal Care and Use Committee of Tulane University.

Enzyme-linked immunosorbent assay

EDTA plasma from SIV+ and SIV+ART animals at pre-infection, 21 dpi, and necropsy or macrophage cell culture supernatants isolated following 24-h exposure to HIV (see cell culture below) were used for quantification of cytokines using enzyme-linked immunosorbent assays (ELISA). ELISAs used in the study were sCD14, total IL-18, caspase-1, high sensitivity IL-1 β (all R&D Systems), and sCD163 (Trillium/IQ Products). All ELISAs were performed in accordance with the manufacturer's instructions and quantified using Gen5 (Biotek). Appropriate standard curve and controls were used in all ELISAs and all samples were tested in duplicate with a CV less than 25% for all values.

Flow cytometry

Flow cytometric analyses were performed with 100 μ L aliquots of EDTA-coagulated whole blood. Erythrocytes were lysed using ImmunoPrep Reagent System (Beckman Coulter), washed twice with PBS containing 2% FBS, and then incubated for 15 min at room temperature with fluorochrome-conjugated surface antibodies. Flow antibody panels were designed to identify cell populations and quantify surface expression. Samples were acquired on a BD FACS Aria (BD Biosciences) and analyzed with Tree Star Flow Jo version 9.6. Monocyte populations were represented as percent of the total monocyte number, while CD4⁺ T cells were represented in absolute number.

Western blot

Tissue samples from SIV-, SIV+, and SIV+ART animals were prepared using mechanical homogenization of the tissues into a lysis buffer containing 6 M Urea, 0.025% SDS, and 5 mM β -mercaptoethanol. Some tissues were extracted from OCT blocks using Trizol (Invitrogen) according to the manufacturer's protocol. Protein assays were performed using Bradford reagent (BioRad). Proteins were resolved on SDS-polyacrylamide gels using BioRad's Western blotting system. The blots were probed with anti-IL-18 (Millipore; 1:1000), anti-caspase 1 (Santa Cruz, clone D3; 1:1000), anti-phosphorylated Serine 536 NF- κ B p65 (Cell Signaling; 1:1000), anti-NF- κ B p65 (Cell Signaling; 1:1000), and anti-actin (Sigma; 1:5000). After three washes with PBS and 0.5% Tween 20 (PBST), the blots were probed with appropriate secondary antibodies and washed in PBST. The signals were detected using ODYSSEY® CLx Imaging system (LI-COR, Inc.). Protein band intensities were quantified using Image Studio Software and normalized against corresponding actin signals.

Monocyte cell culture

Peripheral blood samples were obtained from healthy donors. PBMCs were isolated by Ficoll hypaque (1.0770) gradient centrifugation. Monocytes were separated from other cells by adhesion to 12 well tissue culture plate overnight at 37 °C and 5% CO₂. Wells were

washed with PBS and differentiated to macrophages in RPMI containing 10% FBS and 50 ng/mL M-CSF for 7–10 days. After differentiation, cells were treated with 5 ng/mL HIV virus ADA-M. Cells were infected by spinoculation for 2 h, at 25 °C, 2700 RPMI in 12 well plate with 0.5 mL of inocula per well (Opti-MEM + polybrene 8µg/mL + virus). After spinoculation, cells were incubated for 2 h at 37 °C and 5% CO₂. After a total of 4 h (2 h spinoculation and 2 h incubation), cells were washed three times with 1 mL of PBS and fresh medium containing M-CSF was added. Cells were then incubated for 24 h when cell supernatant was removed for analysis by ELISA.

Statistical analysis

All statistical analyses were performed using Prism Software. A non-parametric, repeat-measures ANOVA was used to compare changes in plasma marker concentrations at multiple time points, and a Kruskal-Wallis ANOVA was used in comparing different groups of the same analysis. If the ANOVA was significant ($P < 0.05$), groups were compared post-hoc using a non-parametric Mann-Whitney *U* test or a non-parametric paired Wilcoxon *t* test. A non-parametric Mann-Whitney *U* test was used to detect variation between two groups. Non-parametric Spearman correlation was used for all correlations. A *P* value of < 0.05 was considered significant for all tests performed.

Results

Caspase-1 pathway activation in the circulation during the course of SIV infection with and without ART

The contribution of HIV-mediated caspase-1 activation and pro-inflammatory cytokines IL-1 β and IL-18 to chronic inflammation in HIV comorbidities remains elusive. To determine the dynamics of the caspase-1 pathway in the circulation during SIV infection, the concentration of active caspase-1, IL-18, and IL-1 β in plasma were measured at pre-infection, 21 dpi (before ART initiation), and necropsy (Fig. 1a–c). No significant difference was found in caspase-1, IL-18, or IL-1 β between the SIV+ and SIV+ART animals before ART initiation. Plasma caspase-1 significantly increased over the course of infection, with dramatic increase from pre-infection to 21 dpi and 21 dpi to necropsy (Fig. 1d). Plasma concentration of IL-18 significantly increased over the course of infection, with an increase from pre-infection to 21 dpi and 21 dpi to necropsy (Fig. 1e). No significant changes were seen in IL-1 β levels during infection (Fig. 1f). The lack of detectable changes in IL-1 β may be due in part to the short half-life of IL-1 β (Kudo et al. 1990).

Within the SIV+ART animals, plasma caspase-1 exhibited a significant spike between pre-infection and 21 dpi, which was significantly decreased with the initiation of ART by comparison of necropsy to peak viral infection (21dpi) (Fig. 1g). IL-18 levels in plasma were increased at 21 dpi and subsequently significantly decrease with ART (Fig. 1h). No significant difference was found between necropsy and preinfection demonstrating ART's ability to restore plasma immune activation if caspase-1 and IL-18 to uninfected levels. There were no significant differences among levels of IL-1 β at any time points (Fig. 1i). To demonstrate effective dampening of the caspase-1 pathway with ART, the percent change of caspase-1, IL-18, and IL-1 β levels from necropsy to 21 dpi were compared. Caspase-1 (Fig.

1j) and IL-18 (Fig. 1k) had a significantly greater percent change in SIV+ animals compared to SIV+ART animals. There was a trend in the percent change of IL-1 β from 21 dpi to necropsy between the two groups (Fig. 1l, $P = 0.1$). Together, these data demonstrate an increase in caspase-1 pathway components in the circulation over the course of SIV infection, which was reversed by the administration of ART.

Caspase-1 pathway activation correlates to monocyte activation and immune cell populations in the circulation of SIV+ animals

Monocytes and macrophages are important reservoirs of virus and contribute to many comorbidities (Kearns et al. 2017a; Burdo et al. 2013; Chivero et al. 2017). We examined the correlation of caspase-1 pathway with monocyte activation markers, monocyte inflammatory subpopulations, and CD4⁺ T cells during SIV infection. Plasma caspase-1, IL-18, and IL-1 β levels at pre-infection, 21 dpi, and necropsy in SIV+ animals were correlated to sCD14, sCD163, CD14⁺CD16⁺ monocytes, and CD4⁺ T cells (Fig. 2). We showed that caspase-1 levels positively correlated with plasma sCD14 (Fig. 2a), sCD163 (Fig. 2b), and the percent of CD14⁺CD16⁺ inflammatory monocytes (Fig. 2c), but negatively correlated with the absolute number of CD4⁺ T cells/ μ L of blood (Fig. 2d). Similarly, plasma IL-18 positively correlated to both plasma sCD14 (Fig. 2e) and sCD163 (Fig. 2f). There was a trend for IL-18 to correlate to the percent of CD14⁺CD16⁺ monocytes (Fig. 2g). IL-18 negatively correlated to the absolute number of CD4⁺ T cells (Fig. 2h). No correlation was found between IL-1 β and any soluble markers of monocyte activation, monocytes, or T cells. These results show an interaction between caspase-1 pathway propagation and monocyte activation, implying a pro-inflammatory effect of caspase-1 and IL-18 on peripheral immune cells. Interestingly, the negative association between CD4⁺ T cell counts and components of the caspase pathway may suggest CD4⁺ T cells as a source of soluble caspase-1 and IL-18 in plasma through pyroptosis (Doitsh et al. 2014).

ART decreases caspase-1 pathway activation and inflammatory markers in the circulation

Next, SIV-associated caspase-1 pathway and immune activation response to ART was evaluated. To evaluate the effects of ART, plasma markers of the caspase-1 pathway and immune cell activation were compared among pre-infection, SIV+, and SIV+ART at necropsy (Table 2). Plasma caspase-1 was significantly different among pre-infection, necropsy of SIV+, and necropsy of SIV+ART animals. Caspase-1 was significantly higher at necropsy in SIV+ animals compared to pre-infection and necropsy in SIV+ART animals (Table 2, $P < 0.01$, $P < 0.05$, respectively). Similarly, the plasma concentration of IL-18 significantly differed between the three groups, with significantly higher IL-18 at necropsy in SIV+ animals compared to levels at pre-infection and at necropsy of SIV+ART animals ($P < 0.001$, $P < 0.01$). There was no significant difference in IL-1 β among the three groups, most likely because of the short half-life of IL-1 β . Plasma sCD14 and sCD163 levels were significantly different among the three time points, with significantly greater sCD14 and sCD163 at necropsy of SIV+ animals compared to pre-infection ($P < 0.05$, $P < 0.01$, respectively). Plasma sCD14 trended toward lower concentrations in ART-treated animals (Table 2; $P = 0.07$), and sCD163 showed a significantly lower concentration in SIV+ART animals compared to SIV+ animals at necropsy ($P < 0.05$). The percent of inflammatory CD14⁺CD16⁺ monocytes exhibited the same trends of increase at necropsy in SIV+ animals

and reduction with ART, although not significant (Table 2, $P = 0.07$). Lastly, there was a significant decrease in the number of CD4⁺ T cells/ μ L of blood in SIV⁺ animals and a return to uninfected levels in the SIV⁺ART animals. These results demonstrate resolved inflammation in the circulation with the implementation of ART, as measured by the decreased caspase-1 pathway, decreased immune activation markers, and recovery of immune cell populations.

ART partially attenuates increase in caspase-1 pathway activation in SIV infection in immune tissues

Previous studies have indicated that ART suppresses inflammation in the circulation, but immune activation may persist in certain tissues in PLWH (Tawakol et al. 2017; Zanni et al. 2016). Therefore, we further investigated how ART affects SIV-associated caspase-1 pathway activation in the lymph node. Necropsy tissue lysates from axillary lymph nodes from SIV⁻, SIV⁺, and SIV⁺ART animals were analyzed by Western blot for caspase-1 and IL-18. Western blots visualized active caspase-1 (25 kD) and pro-caspase-1 (50 kD) to determine the ratio of active caspase-1 to pro-caspase-1 (Fig. 3a). The normalized ratio of active caspase-1 to pro-caspase-1 was significantly different between SIV⁻, SIV⁺, and SIV⁺ART animals, with a significantly increased ratio in the SIV⁺ animals compared to SIV⁻ animals (Fig. 3b). There was no significant difference between the SIV⁺ and SIV⁺ART groups, suggesting the increased activation of caspase-1 during SIV infection was not completely restored with ART. There was no significant difference in active caspase-1 or pro-caspase-1 among the groups (Fig. 3c). Tissue lysates were also analyzed for IL-18 expression to confirm the downstream signaling of the caspase-1 pathway. Blots were analyzed for both pro-IL-18 (25 kD) and active IL-18 (18 kD), as previously reported (Shamaa et al. 2015) (Fig. 3d). Active IL-18 expression in the axillary lymph nodes was significantly increased in both SIV⁺ and SIV⁺ART groups compared to the uninfected controls (Fig. 3e). Interestingly, although ART significantly decreased caspase-1 activation and IL-18 level in the circulation, ART treatment did not decrease IL-18 levels in the lymph nodes. The persistent immune activation in lymph nodes was also seen in the thymus and spleen (Figures S1 and S2). This persistent production of inflammatory mediators in peripheral tissues may be sufficient to drive the residual immune activation seen in PLWH.

The NF- κ B signaling pathway is an important upstream priming signal for activating caspase-1, possibly playing a crucial role for caspase-1 activation in the lymph nodes (Hoseini et al. 2018). HIV infection can trigger immune cell NLRP3 inflammasome formation, caspase-1 activation, and NF- κ B signaling (Chivero et al. 2017; DeLuca et al. 1999; Mayne et al. 1998; Fiume et al. 2012; Shah et al. 2011; Liu et al. 2014; Olivetta et al. 2003; Varin et al. 2003). NF- κ B signaling is a priming event leading the transcriptional up-regulation of NLRP3 inflammasome, pro-IL-1 β , and pro-IL-18. The levels of total NF- κ B (p65) and activated NF- κ B (p-p65) were quantitated by Western blot in lymph nodes from SIV⁻, SIV⁺, and SIV⁺ART animals (Fig. 4a). Both lymph nodes from SIV⁺ and SIV⁺ART rhesus macaques had a significant increase in the ratio of p-p65/p65 compared to SIV⁻ controls (Fig. 4b). The level of p65 was not significantly different between the three groups (Fig. 4c). The level of p-p65 was significantly elevated in lymph nodes of both SIV⁺ and SIV⁺ART animals compared to SIV⁻ controls (Fig. 4d). The increase in p-p65 in SIV

infection and unresolved increase of p-p65 with ART further supports the persistent immune activation in lymphoid tissues.

Caspase-1 increases in human primary macrophages after exposure to HIV

Increase in caspase-1 activation has been documented within 1 day after exposure to and infection by SIV (Barouch et al. 2016). To determine if caspase-1 activation occurs in human macrophages following HIV infection, primary human macrophages were treated with a CCR5-tropic virus ADA-M (5 ng/ mL) for 24 h and cell supernatants were analyzed for caspase-1 and IL-1 β levels. Caspase-1 levels were significantly higher in the supernatant from ADA-M-treated macrophages compared to macrophages not exposed to virus (Fig. 5a, $P<0.001$). After exposure to HIV, IL-1 β levels increased above the level of detection (0.125 pg/mL) compared to untreated cells, where IL-1 β remained below the detection limit (Fig. 5b). These results reaffirm previous findings showing an increase in caspase-1 activation in monocytes/macrophages after HIV infection in vitro (Hernandez et al. 2014; Barouch et al. 2016).

Discussion

The success of ART has dramatically changed the outcome of HIV infection in PLWH. PLWH now have a life expectancy close to that of the general population; however, they still present with a high prevalence of HIV-associated comorbidities including CVD and HAND (Kearns et al. 2017b). Clinical evidence indicates that although ART is able to suppress inflammation in the circulation, immune activation and inflammation may persist in certain tissues (Fitch et al. 2013; Tawakol et al. 2017; Zanni et al. 2016; Pereyra et al. 2012). In the context of viral suppression, the underlying molecular mechanisms connecting HIV-associated immune activation to comorbidities remains largely unknown (Kearns et al. 2017a). In this study, we explored a possible mechanism for HIV-associated immune activation and response to ART in the circulation and immune tissues of SIV-infected rhesus macaques. First, a significant increase in the activation of the caspase-1 pathway was detected in plasma at 21 dpi, with a continued increase at necropsy. Initiation of ART after peak viremia at 21 dpi in SIV-infected animals significantly decreased caspase-1 and IL-18 in the circulation. Markers of immune activation were also decreased in the plasma after early ART, supporting findings of the Strategic Timing of Antiretroviral Therapy (START) study of early ART initiation in PLWH showing decreased immune activation and inflammation (Group 2015; Siedner 2016). Similarly, early intervention with ART in PLWH returned heightened plasma sCD163 levels to levels of uninfected patients (Burdo et al. 2011a); however, in chronically infected PLWH, sCD163 levels remained elevated despite ART. (Burdo et al. 2011a) Our results clearly document that peripheral caspase-1 is contributory to chronic inflammation and disease progression, but is modulated by ART.

The prevalence of HIV comorbidities, with the advent of suppressed viral load, is now a primary focus for disease management. Disease severity in many HIV-associated comorbidities has been associated with specific markers of monocyte/ macrophage activation (Kearns et al. 2017a). Although caspase-1 pathway activation is long-established in HIV, involvement of the caspase-1 cascade in peripheral monocyte/ macrophage immune

activation has never been examined. (Hernandez et al. 2014; Guo et al. 2014; Yearley et al. 2009; Barouch et al. 2016; Burdo et al. 2011b; Lederer et al. 2009) These data indicate caspase-1 activation as a driving mechanism in immune cell activation, thereby contributing to these comorbidities. Positive correlations to monocyte activation markers and inflammatory CD14⁺CD16⁺ monocyte expansion suggest this population may be responsible. Further studies are needed to specifically identify the involvement of caspase-1 and its downstream products not only in modulating monocyte expansion and activation in the periphery but also tissue-specific macrophage activity and polarization. Recently discovered inflammasome activation, the upstream activator of caspase-1, was increased in monocytes from PLWH, and as reaffirmed here, showed that caspase-1 activation was elevated in macrophages after exposure to HIV (Ahmad et al. 2017). Whether caspase-1 activation is required for monocytes/ macrophage activation, leading to these comorbidities, remains unclear. The observed negative correlation of caspase-1 activation to T cell counts further signifies pyroptosis as the main cause of T cell depletion in HIV (Doitsh et al. 2014). Pyroptosis of T cells, and subsequent release of caspase-1, could be a significant source of the increased caspase-1 measured in circulation. The negative association could result from HIV-mediated CD4 T cell depletion, providing a significant enhancement of immune activation. Further experiments will be needed to determine the possible involvement of the caspase-1 pathways in causality of HIV comorbidities. Associations of the caspase-1 pathway to immune cell populations, specifically T cells and macrophages, only begins to shed light on the systemic changes involved in initiating and sustaining chronic immune activation in virally suppressed patients' development of HIV-associated comorbidities.

Notably, caspase-1 pathway activation is different in the tissue compartments compared to the circulation. In the lymph node and spleen, there was significantly greater active caspase-1 in SIV+ animals compared to uninfected controls; however, ART intervention was not able to return to preinfection levels. Tissue immune activation might persist despite adequate suppression of viremia in the circulation as a result of caspase-1 and its effector cytokines, supporting the possible contribution to the pathogenesis of HIV-associated comorbidities. Further, in PLWH, ART is unable to adequately suppress immune activation in tissue compartments (Zanni et al. 2016), suggesting a need for additional therapeutics to target comorbidities directly. Here, we provide evidence that ART is successful in acutely decreasing caspase-1 inflammation in the circulation, but caspase-1 activation persists in tissue compartments. As the life expectancy of PLWH increases, there is an ever-increasing demand to investigate these mechanisms of these chronic comorbidities and develop novel therapeutics for this population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH grants R01 NS082116 (THB), R01CA166144 (XQ), R01 HL130233 (XQ), and R21 AA024984 (XQ), as well as W.W. Smith Charitable Trust A1502 (XQ). The in vivo CD8-depletion antibodies used in these studies were purchased from the NIH Nonhuman Primate Reagent Resource under grants RR016001 and AI040101. We thank Merck and Gilead for the ART drugs used in this study. We would like to thank veterinary staff at the Tulane National Primate Research Center for animal care, and pathology residents and staff for assisting

with necropsies and tissue collection and Dr. Xavier Alvarez and research technician Cecily Midkiff for their assistance on this project. We would like to acknowledge the Tulane National Primate Research Center Tulane's base grant for SIV- tissues and SIVmac251 viral stocks (P510D011104).

References

- Ahmad F, Mishra N, Ahrenstorf G, Franklin BS, Latz E, Schmidt RE, Bossaller L (2017) Evidence of inflammasome activation and for-mation of monocyte derived ASC-specks in HIV-1 positive patients. *AIDS* 32:299–307
- Alcaide ML, Parmigiani A, Pallikkuth S, Roach M, Freguja R, Negra MD, Bolivar H, Fischl MA, Pahwa S (2013) Immune activation in HIV-infected aging women on antiretrovirals-implications for age- associated comorbidities: a cross-sectional pilot study. *PLoS One* 8: e63804
- Barouch DH, Ghneim K, Bosche WJ, Li Y, Berkemeier B, Hull M, Bhattacharyya S, Cameron M, Liu J, Smith K, Borducchi E, Cabral C, Peter L, Brinkman A, Shetty M, Li H, Gittens C, Baker C, Wagner W, Lewis MG, Colantonio A, Kang HJ, Li W, Lifson JD, Piatak M Jr, Sekaly RP (2016) Rapid Inflammasome activation following mucosal SIV infection of rhesus monkeys. *Cell* 165:656–667 [PubMed: 27085913]
- Bryant AK, Moore DJ, Burdo TH, Lakritz JR, Gouaux B, Soontornniyomkij V, Achim CL, Masliah E, Grant I, Levine AJ, Ellis RJ (2017) Plasma soluble CD163 is associated with postmortem brain pathology in human immunodeficiency virus infection. *AIDS (London, England)* 31:973–979
- Burdo TH, Soulas C, Orzechowski K, Button J, Krishnan A, Sugimoto C, Alvarez X, Kuroda MJ, Williams KC (2010) Increased monocyte turnover from bone marrow correlates with severity of SIV encephalitis and CD163 levels in plasma. *PLoS Pathog* 6:e1000842
- Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, Rosenberg ES, Ellis RJ, Williams KC (2011a) Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after anti-retroviral therapy. *J Infect Dis* 204:154–163 [PubMed: 21628670]
- Burdo TH, Lo J, Abbata S, Wei J, DeLelys ME, Pfeffer F, Rosenberg ES, Williams KC, Grinspoon S (2011b) Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. *J Infect Dis* 204:1227–1236 [PubMed: 21917896]
- Burdo TH, Weiffenbach A, Woods SP, Letendre S, Ellis RJ, Williams KC (2013) Elevated sCD163 in plasma but not cerebrospinal fluid is a marker of neurocognitive impairment in HIV infection. *AIDS (London, England)* 27:1387–1395
- Chattergoon MA, Latanich R, Quinn J, Winter ME, Buckheit RW, Blankson JN, Pardoll D, Cox AL (2014) HIV and HCV activate the inflammasome in monocytes and macrophages via endosomal toll-like receptors without induction of type 1 interferon. *PLoS Pathog* 10:e1004082
- Chivero ET, Guo ML, Periyasamy P, Liao K, Callen SE, Buch S (2017) HIV-1 tat primes and activates microglial NLRP3 Inflammasome- mediated neuroinflammation. *J Neurosci* 37:3599–3609 [PubMed: 28270571]
- Crowe SM, Westhorpe CLV, Mukhamedova N, Jaworowski A, Sviridov D, Bukrinsky M (2010) The macrophage: the intersection between HIV infection and atherosclerosis. *J Leukoc Biol* 87:589–598 [PubMed: 19952353]
- Currier JS, Taylor A, Boyd F, Dezii CM, Kawabata H, Burtcel B, Maa JF, Hodder S (2003) Coronary heart disease in HIV-infected individuals. *J Acquir Immune Defic Syndr* 33:506–512 [PubMed: 12869840]
- DeLuca C, Kwon H, Lin R, Wainberg M, Hiscott J (1999) NF-kappaB activation and HIV-1 induced apoptosis. *Cytokine Growth Factor Rev* 10:235–253 [PubMed: 10647779]
- Doitsh G, Galloway NLK, Geng X, Yang Z, Monroe KM, Zepeda O, Hunt PW, Hatano H, Sowinski S, Munoz-Arias I, Greene WC (2014) Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 505:509–514 [PubMed: 24356306]
- Effros RB, Fletcher CV, Gebo K, Halter JB, Hazzard WR, Horne FMF, Huebner RE, Janoff EN, Justice AC, Kuritzkes D, Nayfield SG, Plaeger SF, Schmader KE, Ashworth JR, Campanelli C, Clayton CP, Rada B, Woolard NF, High KP (2008) Aging and infectious diseases: workshop on

- HIV infection and aging: what is known and future research directions. *Clin Infect Dis* 47:542–553 [PubMed: 18627268]
- Fitch KV, Srinivasa S, Abbara S, Burdo TH, Williams KC, Eneh P, Lo J, Grinspoon SK (2013) Noncalcified coronary atherosclerotic plaque and immune activation in HIV-infected women. *J Infect Dis* 208: 1737–1746 [PubMed: 24041790]
- Fiume G, Vecchio E, de Laurentiis A, Trimboli F, Palmieri C, Pisano A, Falcone C, Pontoriero M, Rossi A, Scialdone A, Fasanella Masci F, Scala G, Quinto I (2012) Human immunodeficiency virus-1 Tat activates NF-kappaB via physical interaction with IkappaB-alpha and p65. *Nucleic Acids Res* 40:3548–3562 [PubMed: 22187158]
- Fourman LT, Czerwonka N, Shaikh SD, Stanley TL, Burdo TH, Williams KC, Fitch KV, Lo J, Grinspoon SK (2018) Insulin-like growth factor 1 inversely relates to monocyte/macrophage activation markers in HIV. *AIDS* 32(7):927–932 [PubMed: 29424780]
- Group (2015) I.S.S., et al. initiation of antiretroviral therapy in early asymptomatic HIV infection. *N Engl J Med* 373:795–807 [PubMed: 26192873]
- Guo H, Gao J, Taxman DJ, Ting JP, Su L (2014) HIV-1 infection induces interleukin-1beta production via TLR8 protein-dependent and NLRP3 inflammasome mechanisms in human monocytes. *J Biol Chem* 289:21716–21726 [PubMed: 24939850]
- Guo H, Callaway JB, Ting JP (2015) Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat Med* 21:677–687 [PubMed: 26121197]
- Hasegawa A, Liu H, Ling B, Borda JT, Alvarez X, Sugimoto C, Vinet- Oliphant H, Kim WK, Williams KC, Ribeiro RM, Lackner AA, Veazey RS, Kuroda MJ (2009) The level of monocyte turnover predicts disease progression in the macaque model of AIDS. *Blood* 114:2917–2925 [PubMed: 19383966]
- Hernandez JC, Latz E, Urcuqui-Inchima S (2014) HIV-1 induces the first signal to activate the NLRP3 inflammasome in monocyte-derived macrophages. *Intervirology* 57:36–42 [PubMed: 24008203]
- Hoseini Z, Sepahvand F, Rashidi B, Sahebkar A, Masoudifar A, Mirzaei H (2018) NLRP3 inflammasome: its regulation and involvement in atherosclerosis. *J Cell Physiol* 233:2116–2132 [PubMed: 28345767]
- Kearns A, Gordon J, Burdo TH, Qin X (2017a) HIV-1-associated atherosclerosis: unraveling the missing link. *J Am Coll Cardiol* 69:3084–3098 [PubMed: 28641798]
- Kearns A, Burdo TH, Qin X (2017b) Editorial commentary: clinical management of cardiovascular disease in HIV-infected patients. *Trends Cardiovasc Med* 27:564–566 [PubMed: 28774757]
- Kelesidis T, Kendall MA, Yang OO, Hodis HN, Currier JS (2012) Biomarkers of microbial translocation and macrophage activation: association with progression of subclinical atherosclerosis in HIV-1 infection. *J Infect Dis* 206:1558–1567 [PubMed: 23066162]
- Kudo S, Mizuno K, Hirai Y, Shimizu T (1990) Clearance and tissue distribution of recombinant human interleukin 1 beta in rats. *Cancer Res* 50:5751–5755 [PubMed: 2393849]
- Lakritz JR, Robinson JA, Polydefkis MJ, Miller AD, Burdo TH (2015a) Loss of intraepidermal nerve fiber density during SIV peripheral neuropathy is mediated by monocyte activation and elevated monocyte chemotactic proteins. *J Neuroinflammation* 12:237 [PubMed: 26683323]
- Lakritz JR, Bodair A, Shah N, O'Donnell R, Polydefkis MJ, Miller AD, Burdo TH (2015b) Monocyte traffic, dorsal root ganglion histopathology, and loss of Intraepidermal nerve Fiber density in SIV peripheral neuropathy. *Am J Pathol* 185:1912–1923 [PubMed: 25956030]
- Lederer S, Favre D, Walters KA, Proll S, Kanwar B, Kasakow Z, Baskin CR, Palermo R, McCune JM, Katze MG (2009) Transcriptional profiling in pathogenic and non-pathogenic SIV infections reveals significant distinctions in kinetics and tissue compartmentalization. *PLoS Pathog* 5:e1000296
- Liu X et al. (2014) HIV-1 Nef induces CCL5 production in astrocytes through p38-MAPK and PI3K/Akt pathway and utilizes NF-kB, CEBP and AP-1 transcription factors. *Sci Rep* 4:4450 [PubMed: 24658403]
- Lohse N, Hansen ABE, Pedersen G, Kronborg G, Gerstoft J, Sørensen HT, Væth M, Obel N (2007) Survival of persons with and without HIV infection in Denmark, 1995–2005. *Ann Intern Med* 146:87–95 [PubMed: 17227932]

- Mamik MK et al. (2017) HIV-1 viral protein R activates NLRP3 Inflammasome in microglia: implications for HIV-1 associated Neuroinflammation. *JNeuroimmune Pharmacol* 12:233–248 [PubMed: 27726055]
- Mayne M, Bratanich AC, Chen P, Rana F, Nath A, Power C (1998) HIV-1 tat molecular diversity and induction of TNF-alpha: implications for HIV-induced neurological disease. *Neuroimmunomodulation* 5: 184–192 [PubMed: 9730685]
- Olivetta E, Percario Z, Fiorucci G, Mattia G, Schiavoni I, Dennis C, Jager J, Harris M, Romeo G, Affabris E, Federico M (2003) HIV-1 Nef induces the release of inflammatory factors from human monocyte/ macrophages: involvement of Nef endocytotic signals and NF-kappa B activation. *J Immunol* 170:1716–1727 [PubMed: 12574335]
- Pereyra F, Lo J, Triant VA, Wei J, Buzon MJ, Fitch KV, Hwang J, Campbell JH, Burdo TH, Williams KC, Abbara S, Grinspoon SK (2012) Increased coronary atherosclerosis and immune activation in HIV-1 elite controllers. *AIDS (London, England)* 26:2409–2412
- Porcheray F, Samah B, Leone C, Dereuddre-Bosquet N, Gras G (2006) Macrophage activation and human immunodeficiency virus infection: HIV replication directs macrophages towards a pro-inflammatory phenotype while previous activation modulates macrophage susceptibility to infection and viral production. *Virology* 349:112–120 [PubMed: 16564558]
- Royal W 3rd et al. (2016) Associations between cognition, gender and monocyte activation among HIV infected individuals in Nigeria. *PLoS One* 11:e0147182
- Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K, Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I, Douek DC, INSIGHT SMART Study Group (2011) Plasma levels of soluble CD14 independently predict mortality in HIV infection. *J Infect Dis* 203:780–790 [PubMed: 21252259]
- Saylor D, Dickens AM, Sacktor N, Haughey N, Slusher B, Pletnikov M, Mankowski JL, Brown A, Volsky DJ, McArthur JC (2016) HIV-associated neurocognitive disorder - pathogenesis and prospects for treatment. *Nat Rev Neurol* 12:309 [PubMed: 27080521]
- Shah A, Verma AS, Patel KH, Noel R, Rivera-Amill V, Silverstein PS, Chaudhary S, Bhat HK, Stamatatos L, Singh DP, Buch S, Kumar A (2011) HIV-1 gp120 induces expression of IL-6 through a nuclear factor-kappa B-dependent mechanism: suppression by gp120 specific small interfering RNA. *PLoS One* 6:e21261
- Shamaa OR, Mitra S, Gavrilin MA, Wewers MD (2015) Monocyte Caspase-1 is released in a stable, active high molecular weight complex distinct from the unstable cell lysate-activated Caspase-1. *PLoS One* 10:e0142203
- Shive CL, Jiang W, Anthony DD, Lederman MM (2015) Soluble CD14 is a nonspecific marker of monocyte activation. *AIDS (London, England)* 29:1263–1265
- Siedner MJ (2016) START or SMART? Timing of antiretroviral therapy initiation and cardiovascular risk for people with human immunodeficiency virus infection. *Open Forum Infect Dis* 3:ofw032
- Smith CJ, Ryom L, Weber R, Morlat P, Pradier C, Reiss P, Kowalska JD, de Wit S, Law M, el Sadr W, Kirk O, Friis-Moller N, Monforte A'A, Phillips AN, Sabin CA, Lundgren JD (2014) Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet* 384:241–248 [PubMed: 25042234]
- Subramanian S, Tawakol A, Burdo TH, Abbara S, Wei J, Vijayakumar J, Corsini E, Abdelbaky A, Zanni MV, Hoffmann U, Williams KC, Lo J, Grinspoon SK (2012) Arterial inflammation in patients with HIV. *JAMA* 308:379–386 [PubMed: 22820791]
- Tawakol A, Ishai A, Li D, Takx RA, Hur S, Kaiser Y, Pampaloni M, Rupert A, Hsu D, Sereti I, Fromentin R, Chomont N, Ganz P, Deeks SG, Hsue PY (2017) Association of arterial and lymph node inflammation with distinct inflammatory pathways in human immunodeficiency virus infection. *JAMA Cardiol* 2(2):163–171 [PubMed: 27926762]
- Tenorio AR, Zheng Y, Bosch RJ, Krishnan S, Rodriguez B, Hunt PW, Plants J, Seth A, Wilson CC, Deeks SG, Lederman MM, Landay AL (2014) Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment. *J Infect Dis* 210:1248–1259 [PubMed: 24795473]

- Triant VA, Lee H, Hadigan C, Grinspoon SK (2007) Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab* 92:2506–2512 [PubMed: 17456578]
- Varin A, Manna SK, Quivy V, Decrion AZ, van Lint C, Herbein G, Aggarwal BB (2003) Exogenous Nef protein activates NF-kappa B, AP-1, and c-Jun N-terminal kinase and stimulates HIV transcription in promonocytic cells. Role in AIDS pathogenesis. *J Biol Chem* 278:2219–2227 [PubMed: 12419805]
- Walker JA, Sulciner ML, Nowicki KD, Miller AD, Burdo TH, Williams KC (2014) Elevated numbers of CD163+ macrophages in hearts of simian immunodeficiency virus-infected monkeys correlate with cardiac pathology and fibrosis. *AIDS Res Hum Retrovir* 30:685–694 [PubMed: 24524407]
- Walsh JG, Reinke SN, Mamik MK, McKenzie BA, Maingat F, Branton WG, Broadhurst DI, Power C (2014) Rapid inflammasome activation in microglia contributes to brain disease in HIV/AIDS. *Retrovirology* 11:35 [PubMed: 24886384]
- Williams K, Burdo TH (2012) Monocyte mobilization, activation markers, and unique macrophage populations in the brain: observations from SIV infected monkeys are informative with regard to pathogenic mechanisms of HIV infection in humans. *J NeuroImmune Pharmacol* 7:363–371 [PubMed: 22167311]
- Yearley JH, Xia D, Pearson CB, Carville A, Shannon RP, Mansfield KG (2009) Interleukin-18 predicts atherosclerosis progression in SIV-infected and uninfected rhesus monkeys (*Macaca mulatta*) on a high-fat/high-cholesterol diet. *Lab Invest* 89:657–667 [PubMed: 19381133]
- Zanni MV, Toribio M, Robbins GK, Burdo TH, Lu MT, Ishai AE, Feldpausch MN, Martin A, Melbourne K, Triant VA, Suchindran S, Lee H, Hoffmann U, Williams KC, Tawakol A, Grinspoon SK (2016) Effects of antiretroviral therapy on immune function and arterial inflammation in treatment-naïve patients with human immunodeficiency virus infection. *JAMA Cardiol* 1:474–480 [PubMed: 27438325]

Fig. 1.

Caspase-1 pathway activation in plasma over the course of SIV infection with and without ART. Plasma caspase-1 (a, d, g), IL-18 (b, e, h), and IL-1 β (c, f, i) were measured at pre-infection (Pre), 21 days postinfection (21 dpi), and at necropsy (necropsy) from 7 SIV-infected (denoted SIV+, black) and 6 SIV-infected ART-treated (denoted SIV+ART, red) rhesus macaques. The means and the standard error of the means (SEM) are shown. ART treatment was initiated following a pre-treatment blood draw on 21 dpi (a-c). Longitudinal analysis of plasma markers is shown for each animal, SIV+ and SIV+ART (d-i). d Plasma caspase-1 in SIV+ monkeys ($n = 7$) was significantly increased during the course of SIV infection (ANOVA $P < 0.001$). Caspase-1 was elevated at 21 dpi ($P < 0.05$) and necropsy ($P <$

0.05) compared to pre-infection levels. **e** Plasma IL-18 in SIV+ monkeys ($n = 7$) was significantly increased during the course of SIV infection (ANOVA $P < 0.01$), with significant difference between pre-infection and 21 dpi ($P < 0.05$) and 21 dpi and necropsy ($P < 0.01$). **f** Plasma IL-1 β in SIV+ monkeys ($n = 7$) trended toward an increase during the course of SIV infection. **g** Plasma caspase-1 in SIV+ ART animals ($n = 6$) was significantly altered during the course of SIV infection and treatment (ANOVA $P < 0.01$). Caspase-1 was elevated at 21 dpi ($P < 0.05$) compared to pre-infection level and significantly decreased with ART, compared by necropsy to 21 dpi ($P < 0.05$). **h** Plasma IL-18 in SIV+ART animals ($n = 6$) was significantly altered during the course of SIV infection and treatment (ANOVA $P < 0.01$) and was significantly decrease from 21 dpi to necropsy after ART ($P < 0.01$). **i** Plasma IL-1 β in SIV+ART animals ($n = 6$) was not significantly altered during the course of SIV infection or with treatment. The percent change (mean and SEM) in caspase-1 (**j**), IL-18 (**k**), and IL-1 β (**l**) levels from 21 dpi to necropsy was calculated in SIV+ and SIV+ART animals. **j** There was a significant difference in the percent change of caspase-1 in the SIV + (49.8%) compared to the SIV+ART (-48.8%) animals ($P < 0.05$). **k** There was a significant difference in the percent change of IL-18 in the SIV+ (231.1%) compared to the SIV+ART (-66.1%) animals ($P < 0.01$). **l** There was a trend in the difference in percent change of IL-1 β from 21 dpi to necropsy between the two groups (SIV+ 202.6%, SIV+ART -5.4%; $P = 0.1$). Longitudinal concentration of caspase-1 pathway activation markers was compared using a repeated-measures, non-parametric ANOVA and, if significant ($P < 0.05$), Wilcoxon non-parametric paired post-hoc analysis was used to determine significant difference between the groups. The percent change was compared using a Mann-Whitney, two-tailed t test. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Fig. 2.

Caspase-1 pathway activation correlates to monocyte activation and immune cell populations. Plasma caspase-1 (**a-d**) and IL-18 (**e-h**) were correlated to plasma sCD14 (**a, e**), plasma sCD163 (**b, f**), the percent of CD14⁺CD16⁺ monocytes (MC) (**c, g**), and the absolute number of CD4⁺ T cells (**d, h**) at pre-infection, 21 dpi, and necropsy in SIV+ animals.

Caspase-1 levels positively correlated with sCD14 (**a**: $P = 0.0099$; $R = 0.55$) and sCD163 (**b**: $P = 0.033$; $R = 0.47$), CD14⁺CD16⁺ monocytes (**c**: $P = 0.04$; $R = 0.49$). Caspase-1 and the absolute number of CD4⁺ T cells negatively correlated (**d**: $P = 0.019$; $R = -0.68$). IL-18 positively correlated to both sCD14 (**e**: $P = 0.0002$; $R = 0.72$) and sCD163 (**f**: $P = 0.008$; $R = 0.56$). IL-18 trended toward correlation to the percent of CD14⁺CD16⁺ monocytes (**g**). IL-18 and the absolute number of CD4⁺ T cells negatively correlated (**h**: $P = 0.028$; $R = -0.64$).

All correlations were compared by non-parametric Spearman correlation

Fig. 3.

Caspase-1 pathway activation persists in lymph node despite ART. Tissue lysates from axillary lymph nodes were analyzed by western blot for caspase-1 (**a-c**) and IL-18 (**d, e**). **a** Blots were visualized for pro-caspase-1 (50 kD), active caspase-1 (25 kD), and β-actin. β-actin was used for a loading control and all samples were normalized to β-actin. **b** The ratio of active caspase to pro-caspase was compared between groups. There was a significant difference between groups (ANOVA $P < 0.05$). Caspase-1 activation significantly increased in the lymph node following SIV infection ($P < 0.05$). Although ART decreased caspase-1 activation, it was still not significantly different from SIV infection. **c** Pro-caspase-1 and caspase-1 were not significant between groups. **d** Blots were visualized for pro-IL-18 (24 kD), active IL-18 (18 kD), and β-actin. β-actin was used for a loading control and all samples were normalized to β-actin. **e** There was a significant difference among the levels of active IL-18 in lymph nodes from SIV-, SIV+, and SIV+ART animals (ANOVA $P < 0.05$).

Active IL-18 was significantly increased in the lymph node with SIV infection compared to SIV- ($P < 0.05$). Additionally, after ART IL-18 remained elevated compared to SIV- controls ($P < 0.05$). Groups were compared using a non-parametric Kruskal- Wallis ANOVA and, if significant ($P < 0.05$), a non-parametric MannWhitney post hoc comparison was used. (* $P < 0.05$)

Fig. 4.

NF-κB pathway activation in lymph node despite ART. Tissue lysates from axillary lymph nodes were analyzed by western blot for NF-κB molecules (**a-d**). **a** Blots were visualized for total NF-κB (p65), phosphorylated NF-κB (Ser536)(p-p65), and β-actin in lymph nodes of SIV-, SIV+, and SIV+ART animals. β-actin was used for a loading control and all samples were normalized to β-actin. **b** There was a significant difference in the ratio of p-p65/p65 among the three groups ($P = 0.05$). Both lymph nodes from SIV+ and SIV+ART rhesus macaques had a significant increase in the ratio of p-p65/p65 compared to SIV- controls (ANOVA < 0.05 ; $P < 0.05$). **c** There was a trend for a difference in p65 among the three groups ($P = 0.05$). **d** There was a significant difference in the level of p-p65 among the three groups ($P = 0.05$). Both lymph nodes from SIV+ and SIV+ART rhesus macaques had a significant increase in p-p65 levels compared to SIV- controls (ANOVA < 0.05 ; $P < 0.05$). Groups were compared using a non-parametric Kruskal-Wallis ANOVA and, if significant ($P < 0.05$), a non-parametric Mann-Whitney post-hoc comparison was used. (* $P < 0.05$)

Fig. 5.

Caspase-1 increases in macrophages after only 24 h from exposure to HIV. **a, b** Primary human macrophages were treated with CCR5 virus ADA-M (5 ng/mL virus) for 24 h and then cell supernatant was analyzed for caspase-1 activation (**a**) and IL-1 β levels (**b**). **a** Caspase-1 levels were significantly higher in ADA-M-treated macrophages compared to macrophages not exposed to virus (No TX: 56.62 $\pm$ 2.0 pg/mL vs. ADA-M 113.2 $\pm$ 3.9 pg/mL, $P < 0.001$, $n = 3$). Comparison between virus-treated and untreated cells was done using an unpaired t test. **b** After exposure to HIV, IL-1 β levels increased above the level of detection (0.174 $\pm$ 0.04 pg/ mL) compared to untreated cells, where IL-1 β was below the detection limit (0.125 pg/mL). IL-1 β was unable to be statistically compared due to levels falling below detection. (***) $P < 0.001$, *N.D.* not detectable)

Table 1

Viral, drug, and zoological information of animals used in the study

Animal No.	Infection status	Drug admin.	CD8 depletion	Survival (days)	Species	Sex	Age (years)	Term. viral load (Log10)
A01	SIV-	N/A	N/A	N/A	RM	M	3.28	N/A
A02	SIV-	N/A	N/A	N/A	RM	M	3.41	N/A
A03	SIV-	N/A	N/A	N/A	RM	M	2.27	N/A
A04	SIV	N/A	Depleted	55	RM	M	7.3	7.83
A05	SIV	N/A	Depleted	89	RM	M	10.4	7.71
A06	SIV	N/A	Depleted	174	RM	M	10.8	7.28
A07	SIV	N/A	Depleted	146	RM	M	11.5	7.67
A08	SIV	N/A	Depleted	84	RM	M	6.3	5.41
A09	SIV	N/A	Depleted	106	RM	M	4.5	7.69
A10	SIV	N/A	Depleted	96	RM	M	7.3	7.15
A11	SIV	ART	Depleted	120	RM	M	10.4	2.87
A12	SIV	ART	Depleted	120	RM	M	6.2	2.34
A13	SIV	ART	Depleted	119	RM	M	10.3	3.62
A14	SIV	ART	Depleted	119	RM	M	6.4	1.60
A15	SIV	ART	Depleted	118	RM	M	6.7	2.66
A16	SIV	ART	Depleted	118	RM	M	6.1	2.66

N/A not applicable, No. number, Admin. administration, SIV- uninfected, SIV+ SIV infected, ART ART treated, RM Rhesus Macaque, M male, Term. Terminal

Table 2
ART-treatment decreases caspase-1 pathway activation and inflammatory markers in circulation

	Pre-infection (Pre)			SIV+ (+)			SIV+ART (ART)			ANOVA ^a		Post-hoc	
	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.	n	P		Pre/+	+ /ART
Caspase-1 (pg/mL)	112.9	22.3	13	343.3	48.3	7	120.7	48.9	6	0.006		**	*
IL-18 (pg/mL)	186.0	36.1	13	1561.4	769.0	7	165.4	39.3	6	0.001		***	**
IL-1 β (pg/mL)	1.1	0.3	13	3.3	1.2	7	1.0	0.8	6	ns		nd	nd
sCD14 (ng/mL)	791.0	42.0	13	1293.6	156.5	7	767.4	99.6	6	0.028		*	ns
sCD163 (ng/mL)	283.1	18.9	13	549.5	117.0	7	283.9	28.9	6	0.019		**	*
CD14 ⁺ CD16 ⁺ Monocytes (%)	10.3	1.3	10	24.0	6.7	4	6.0	1.2	6	0.049		ns	ns
CD4 ⁺ T cells (per μ L blood)	963.1	173.1	10	263.7	64.4	4	1101.5	209.3	6	0.009		**	**

S.E. standard error, n number, ANOVA analysis of variance, nd not done, ns not significant

^aIf ANOVA had $p < 0.05$, a post-hoc Mann-Whitney test compared pre-infection (Pre) to necropsy SIV+ (+) and necropsy SIV+ ART (ART) values.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$