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Suppressed Th17 Levels Correlate with Elevated PIAS3, SHP2, and SOCS3 Expression in CD4 T Cells during Acute Simian Immunodeficiency Virus Infection

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T helper 17 (Th17) cells play an important role in mucosal immune homeostasis and maintaining the integrity of the mucosal epithelial barrier. Loss of Th17 cells has been extensively documented during human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infections. The lack of effective repopulation of Th17 cells has been associated with chronic immune activation mediated by the translocation of microbial products. Using *ex vivo* analysis of purified peripheral blood CD4 T cells from SIV-infected rhesus macaques, we show that the suppression of interleukin-17 (IL-17) expression correlated with upregulated expression of negative regulatory genes PIAS3, SHP2, and SOCS3 in CD4 T cells. Suppressed Th17 expression was accompanied by elevated levels of soluble CD14 (sCD14) and lipopolysaccharide binding protein (LBP) in the plasma during early stages of infection. Plasma viral loads rather than sCD14 or LBP levels correlated with acute immune activation. Additionally, we observed a significant increase in the expression of CD14 on peripheral blood monocytes that correlated with IL-23 expression and markers of microbial translocation. Taken together, our results provide new insights into the early events associated with acute SIV pathogenesis and suggest additional mechanisms playing a role in suppression of Th17 cells.

Progressive human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infections are characterized by chronic immune activation (1) that is partially mediated by translocated microbial products (2). Microbial translocation has been correlated with the loss of T helper 17 (Th17) cells, and numerous studies have documented the loss of Th17 cells during early SIV infection (3, 4). Loss of Th17 cells was associated with translocation of *Salmonella* into the systemic circulation (5). Others (3, 4, 6, 7) have shown that the loss of interleukin-17 (IL-17)-producing cells accompanied mucosal damage and translocation of bacterial products during pathogenic SIV infections. Favre et al. (4) demonstrated that the loss of Th17 cells was accompanied by systemic T cell activation. In contrast, nonpathogenic infection of sooty mangabeys was associated with preservation of the Th17 cells and mucosal barrier integrity (6, 8), whereas Ciccone et al. (9) showed that Th17 cells were preserved in HIV-infected long-term nonprogressors.

Similar to chronic infection, a high level of immune activation has also been observed during acute SIV infection (10–13). It is not clear if microbial products translocate early in infection and if these translocated products contribute to immune activation during acute infection. We sought to address this question by examining the markers of microbial translocation and immune activation in rhesus macaques during acute SIV infection and correlated these with Th17 expression. Our results showed that markers of microbial translocation were significantly upregulated in the plasma of SIV-infected animals very early during the course of infection and that this was accompanied by a loss of IL-17 expression in CD4 T cells. Interestingly, plasma viral loads rather than IL-17 expression or markers of microbial translocation positively correlated with $CD8^+$ $KI\text{-}67^+$ T cells, suggesting that acute viral replication rather than translocated bacterial products likely plays a primary role in acute immune activation. Suppressed IL-17 expression correlated with upregulated expression of various negative regulatory genes, such as PIAS3, SHP2, and SOCS3, indicating a potential role for these genes in suppressing Th17 responses during SIV infection.

MATERIALS AND METHODS

Animals, infection, and samples. Rhesus macaques (*Macaca mulatta*) of Indian origin ($n = 6$) were used for this study. The animals were housed in accordance with the American Association for Accreditation of Laboratory Animal Care guidelines and were seronegative for SIV, simian retrovirus (SRV), and simian T-cell leukemia virus (STLV) type 1. Animals were infected intravenously with uncloned pathogenic SIVmac251.

Peripheral blood was collected at various time points and used for isolation of plasma and peripheral blood mononuclear cells (PBMCs). PBMCs were isolated by density gradient centrifugation and cryopreserved. Plasma viral loads were determined by real-time PCR using reverse-transcribed viral RNA as the template, as previously described (14). Commercial enzyme-linked immunosorbent assay kits were used to measure plasma soluble CD14 (sCD14; R&D Systems) and lipopolysaccharide (LPS) binding protein (LBP; Cell Sciences) levels as per the manufacturer's instruction.

Antibodies and flow cytometry. Isolated cells were labeled with specific panels of antibodies containing different combinations of CD3-Cy7-allophycocyanin (APC), CD4-Pacific Blue (PB), CD28-Cy5-phycoerythrin (PE), CD95-fluorescein isothiocyanate (FITC), and CD8-Alexa Fluor 700 or CD3-Cy7-APC, CD4-PB, CD28-Cy5-PE, CD95-APC, CD8-Alexa Fluor 700, and Ki-67–FITC and analyzed by flow cytometry as described previously (15–21). All the antibodies were obtained from BD Biosciences (San Diego, CA) and titrated using rhesus macaque PBMCs. Ki-67 expression in CD8 memory T cells was determined by intracellular staining using a Cytofix/Cytoperm kit from BD Biosciences. Labeled cells were

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fixed with 0.5% paraformaldehyde and analyzed using a Becton, Dickinson LSR II flow cytometer.

For analysis of IL-17, STAT3 (signal transducer and activator of transcription 3), PIAS3 (protein inhibitor of activated STAT3), SHP2 (Src homology region 2 domain-containing phosphatase 2), and SOCS3 (suppressor of cytokine signaling 3) mRNA expression, total viable peripheral blood CD4 T cells from days 7 and 35 postinfection (p.i.) were sorted by negative selection using a BD FACSAria sorter. We did not have sufficient samples from the other time points; hence, we were unable to include these samples in our analysis. To avoid signaling and activation through CD3 and CD4, cells were labeled only with anti-CD14, anti-CD8, anti-CD20, and the live/dead amine-reactive dye VIVID. VIVID-positive dead cells were excluded along with $CD14^+$, $CD8^+$, and $CD20^+$ cells. An aliquot of the negatively sorted cells was labeled with anti-CD3 and anti-CD4 to determine purity and enrichment of the sorted subset. Approximately 5×10^5 $CD4^+$ T cells/well were seeded in 96-well tissue culture plates and stimulated with 40 units/ml of recombinant human interleukin-6 (rIL-6; R&D Systems) in serum-free RPMI 1640 for 15 min at 37°C, as previously described for rhesus macaques (22). Unstimulated cells were set up simultaneously as controls. After 15 min, cells were harvested, washed, and used for RNA isolation. For *ex vivo* analysis of Toll-like receptor 4 (TLR4) and IL-23 mRNA in monocytes, $CD14^+$ monocytes were positively sorted from PBMCs at days 7 and 35 p.i. and used *ex vivo* for RNA isolation.

Relative qPCR assay. RNA was isolated using an RNeasy kit (Qiagen Sciences, Gaithersburg, MD) and treated with Ambion Turbo DNase (Applied Biosystems, Austin, TX) to remove contaminating DNA. Each DNase-treated RNA sample was tested using a quantitative PCR (qPCR) assay with the β-actin primers described below to confirm that RNA was free from DNA contamination. Purified RNA was reverse transcribed with a SuperScript III first-strand synthesis kit (Invitrogen, Carlsbad, CA) to synthesize cDNA that was used to determine the expression of genes using an ABI 7500 instrument (Applied Biosystems). TaqMan qPCR was performed using high-fidelity Platinum *Taq* polymerase (Invitrogen) as described previously with *Macaca mulatta*-specific (i) IL-17 primers IL-17-F (ACCAATCCCAAAAGGTCCTC) and IL-17-R (TCTCTCAGGGT CCTCATTGC) and probe IL-17-P (FAM-CAACCGATCCACCTCACCT TGG-BHQ1, where FAM is 6-carboxyfluoresein and BHQ1 is black hole quencher 1), (ii) STAT3 primers STAT3-F (GGAGGAGTTGCAGCAAA AAG) and STAT3-R (GATTCTCTCCTCCAGCATCG) and probe STAT3-P (FAM-CCCCATTGTACAGCACCGGC-BHQ1), (iii) PIAS3 primers PIAS3-F (ACCATTGCCCTTCTATGAAGTC) and PIAS3-R (AG GTAAAGTGCGCTTCCTCA) and probe PIAS3-P (FAM-ACCACCCTT GCATCCACTTCTA-BHQ1), (iv) SHP2 primers SHP2-F (ATATGGCG TCATGCGTGTTA) and SHP2-R (TCCGTATTCCCTTGTCCAAC) and probe SHP2-P (FAM-TGTCAAAGAAAGTGCTGCTCATGA-BHQ1), (v) SOCS3 primers SOCS3-F (TTCTACTGGAGCGCAGTGAC) and SOCS3-R (CTGTCGCGAATCAGAAAGGT) and probe SOCS3-P (FAM-AGGCGAACCTGCTGCTCAGC-BHQ1) (vi) IL-6 primers IL-6-F (ATG CAATAACCACCCCTGAA) and IL-6-R (AAGAGCCCTCAGGTTGG ACT) and probe IL-6-P (FAM-TGCTGACGAAGCTGCAGGCA-BHQ 1), (vii) IL-21 primers IL-21-F (TGTGAATGACTTGGACCCTGAA) and IL-21-R (AAACAGGAAATAGCTGACCACTCA) and probe IL-21-P (FA M-TCTGCCAGCTCCAGAAGATGTAGAGACAAACT-BHQ1), (viii) IL-23p19 primers IL-23-F (CCAGCAGCTTTCACAGAAGC) and IL-23-R (TCTTAGATCCATGTGTCCCACT) and probe IL-23-P (FAM-TG GCCTGGAGTGCACATCCA-BHQ1), (ix) transforming growth factor β (TGF-β) primers TGFb-F (TGTCATAGATTTCGTTGTGGGTTT) and TGFb-R (GTACAACAGCACCCGCGAC) and probe TGFb-P (FAM-AC CATTAGCACGCGGGTGACCTCC-BHQ1), and (x) TLR4 primers TLR4-F (CCTTTCAGCTCTGCCTTCAC) and TLR4-R (CACCTTTCGG CTTTTATGGA) and probe TLR4-P (FAM-ATTCCCGGTGTGGCCATT GC-BHQ1); and gene expression was normalized to *Macaca mulatta* β-actin housekeeping gene expression using β-actin-specific primers β-Actin-F (ATGCTTCTAGGCGGACTGTG) and β-Actin-R (AAAGCC ATGCCAATCTCATC) and probe β-Actin-P (FAM-TGCGTTACACCC TTTCTTGACAAAACC-BHQ1). Primers and probes were designed using Primer-3 software (23). Collected data were analyzed using the $2^{-\Delta\Delta CT}$ (ddC_T, where C_T is the threshold cycle) method with ABI 7500 software, and fold changes in gene expression levels were calculated as previously described (24).

Data analysis. Flow cytometric data were analyzed using FlowJo (version 9.2) software (Tree Star, Inc., Ashland, OR). Statistical analysis was performed using the *t* test with GraphPad Prism (version 4.0) software (GraphPad Prism Software, Inc. San Diego, CA). A P value of <0.05 was considered significant. Error bars represent standard errors. Linear regression analysis was performed to determine line of fit, correlations were derived using the Spearman correlation, and a P value of <0.05 was considered significant.

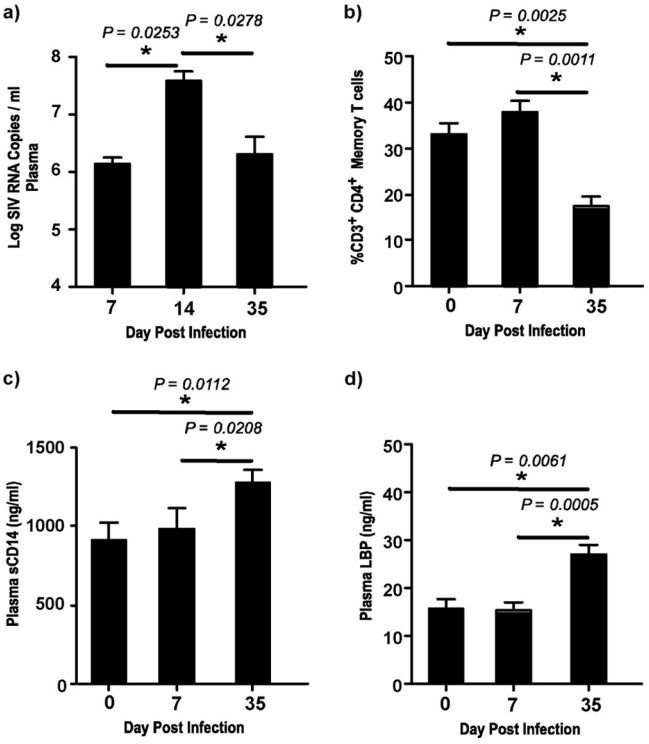

FIG 1 Plasma sCD14 and LBP levels are significantly elevated during acute SIV infection. (a) Plasma viral loads at days 7, 14, and 35 p.i. The limit of detection was 30 copies/ml of plasma. (b) Percentage of total $CD3^+$ $CD4^+$ memory T cells in peripheral blood at days 0, 7, and 35 p.i. Memory CD4 T cells were identified on the basis of the expression of CD28 and CD95, with all memory CD4 T cells expressing CD95. (c and d) Plasma sCD14 (c) and plasma LBP (d) levels at days 0, 7, and 35 p.i.

RESULTS

Plasma sCD14 and LBP levels are significantly elevated during acute SIV infection. Acute SIV infection has been associated with the loss of memory CD4 T cells in peripheral blood (10, 19, 25). To confirm these findings, we first evaluated plasma viral loads (Fig. 1a) and the levels of memory CD4 T cells (Fig. 1b) at days 0, 7, 14, and 35 p.i. Plasma viremia peaked at day 14 p.i., followed by a significant decline to a set point. In line with previous reports, the decline in plasma viral loads was accompanied by a significant loss of memory CD4 T cells at day 35 p.i. compared to the levels at days 0 and 7 p.i. (10, 19, 25).

Next, we examined the plasma levels of sCD14 (Fig. 1c) and LBP (Fig. 1d) at day 35 p.i. and compared them to those at days 0

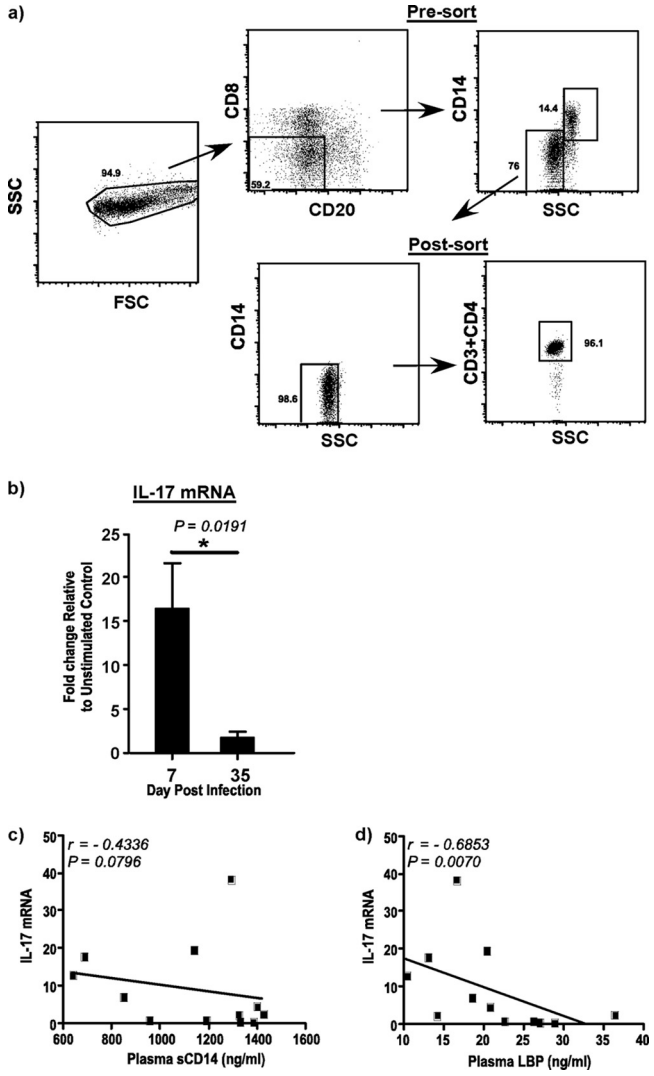

FIG 2 IL-17 expression in CD4 T cells is significantly suppressed during acute SIV infection. (a) Gating strategy used to sort pure populations of peripheral blood CD4 T cells by fluorescence-activated cell sorting. PBMCs were labeled with anti-CD20, anti-CD8, and anti-CD14 and negatively sorted for CD4 T cells. An aliquot of the sorted cells was stained with anti-CD4 to confirm purity. SSC, side scatter. (b) IL-17 mRNA expression in purified peripheral blood CD4 T cells after short-term stimulation for 15 min with recombinant human IL-6. The fold change at days 7 and 35 p.i. relative to the levels for unstimulated control cells is shown. (c and d) IL-17 expression negatively correlates with LBP (c) and sCD14 (d) levels in plasma.

and 7 p.i. Soluble CD14 and LBP have been extensively used as markers of microbial translocation (2, 26–28). We observed a significant increase in the plasma levels of both sCD14 and LBP at day 35 p.i. compared to those at the earlier time points, suggesting that early viral replication and CD4 T cell loss were accompanied by an increase in the markers of microbial translocation.

IL-17 expression is significantly suppressed during acute SIV infection and negatively correlates with sCD14 and LBP levels. To determine if the increased levels of markers of microbial translocation were accompanied by a loss of Th17 cells, we evaluated the *ex vivo* expression of IL-17 in purified populations of negatively sorted CD4 T cells (Fig. 2a) following short-term stimulation with IL-6 at days 7 and 35 p.i. Previous studies have shown that both the Th17 compartment (19) and the total memory CD4 T cell compartment (10, 25) were largely intact during the first 7 to 10 days after infection. Our results showed that IL-17 expression was significantly suppressed at day 35 p.i. compared to that at day 7 p.i. (Fig. 2b).

Next, we correlated IL-17 expression with the levels of plasma sCD14 (Fig. 2c) and LBP (Fig. 2d) to examine if suppressed IL-17 expression was associated with elevated levels of these markers of microbial translocation. Soluble CC14 levels were found to negatively correlate ($r = -0.4336$; $P = 0.0796$) with IL-17 expression, though this correlation was not significant. In contrast, LBP levels were found to have a significantly high negative correlation ($r = -0.6853$; $P = 0.0070$) with IL-17 expression levels in CD4 T cells.

Upregulated PIAS3, SHP2, and SOCS3 mRNA levels correlate with suppression of IL-17 expression. IL-17 production is negatively regulated by a number of intracellular factors, such as PIAS3, SHP2, and SOCS3 (29, 30). To determine if the suppression of IL-17 expression during acute SIV infection was due to negative regulation, we evaluated the expression of PIAS3, SHP2, and SOCS3 mRNA (Fig. 3a) in purified populations of negatively sorted CD4 T cells at day 35 p.i. after short-term stimulation with IL-6 and compared it to that at day 7 p.i. We observed a significant upregulation in the expression of PIAS3, SHP2, and SOCS3 at day 35 p.i. compared to that at day 7 p.i.

Next, we correlated the expression of PIAS3 (Fig. 3b), SHP2 (Fig. 3c), and SOCS3 (Fig. 3d) with the expression of IL-17. PIAS3 ($r = -0.6434$; $P = 0.0120$), SHP2 ($r = -0.5804$; $P = 0.0239$), and SOCS3 ($r = -0.5315$; $P = 0.0377$) levels had a significantly high negative correlation with IL-17 expression, suggesting that these negative regulators may contribute to the suppression of IL-17 expression during acute SIV infection.

Plasma sCD14 and LBP levels significantly correlate with IL-23 expression in CD14$^+$ monocytes. A number of cytokines, such as IL-6, IL-21, IL-23, and TGF-β, have been shown to be critical for the induction of IL-17 expression in CD4 T cells (31–33). To determine if suppressed IL-17 expression in CD4 T cells at day 35 p.i. was due to the paucity of IL-17-promoting cytokines, we evaluated the expression of IL-6, IL-21, IL-23, and TGF-β mRNA *ex vivo* in total PBMCs at day 7 p.i. and compared it to that at day 35 p.i. (Fig. 4a). There were no major changes in the expression levels of IL-6, IL-21, and TGF-β at day 35 p.i. relative to day 7 p.i. However, the expression of IL-23 was significantly elevated at day 35 p.i. compared to that at day 7 p.i.

As IL-23 is primarily secreted by myeloid cells, we examined the *ex vivo* expression of IL-23 in purified populations of CD14$^+$ monocytes from PBMCs at days 7 and 35 p.i. (Fig. 4b and c). Our results showed that IL-23 levels were significantly elevated in CD14$^+$ monocytes at day 35 p.i. compared to day 7 p.i.

Previous studies have shown that translocated microbial products could activate blood monocytes (34). To determine if translocated microbial products could potentially activate monocytes, we evaluated the expression of TLR4 mRNA by quantitative reverse transcription-PCR (Fig. 4c) in purified populations of CD14$^+$ monocytes and determined the density of CD14 expression on monocytes by flow cytometry (Fig. 4d) at day 7 p.i. and compared it to that at day 35 p.i. Both TLR4 and CD14 are primary receptors for LPS. There was no major change in the levels of TLR4 expression in monocytes. However, the density of CD14 expression was significantly upregulated on monocytes at day 35 p.i. compared to day 7 p.i.

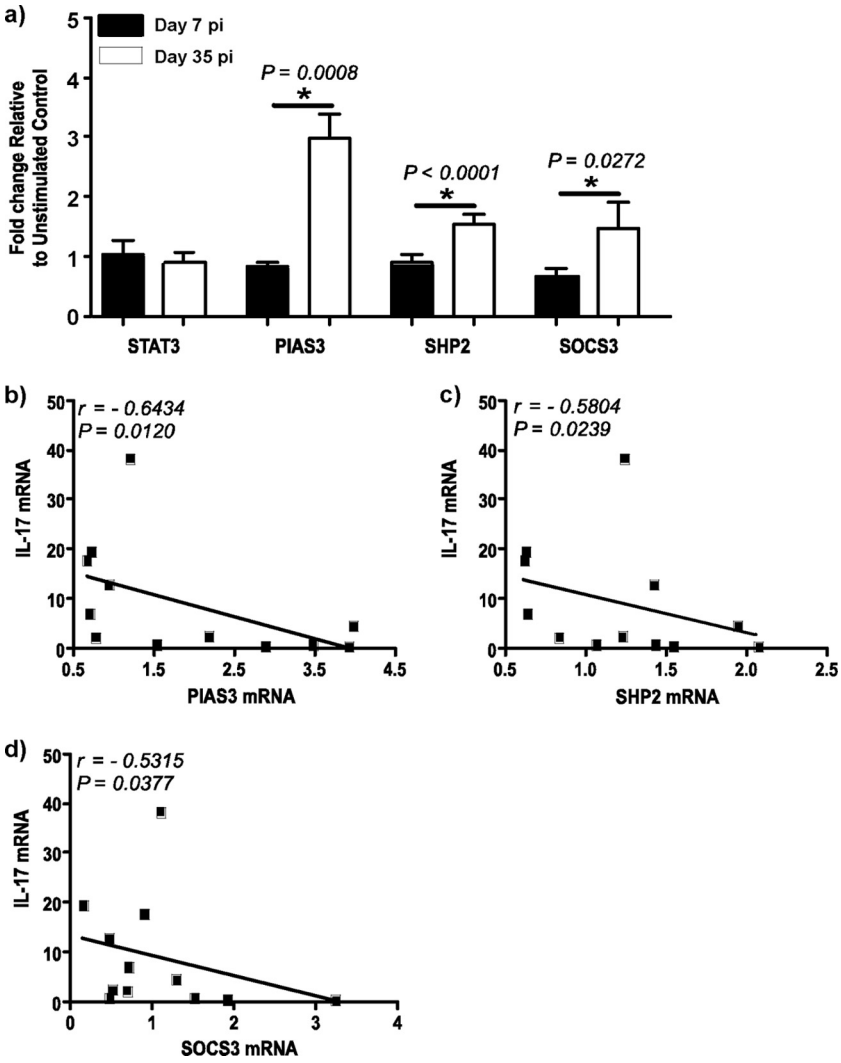

FIG 3 Expression of PIAS3, SHP2, and SOCS3 is significantly upregulated in CD4 T cells during acute SIV infection. (a) STAT3, PIAS3, SHP2, and SOCS3 mRNA expression in purified peripheral blood CD4 T cells at days 7 and 35 p.i. after short-term stimulation for 15 min with recombinant human IL-6. The fold change at days 7 and 35 p.i. relative to the levels for unstimulated control cells is shown. (b to d) IL-17 expression negatively correlates with PIAS3 (b), SHP2 (c), and SOCS3 (d) levels in peripheral blood CD4 T cells.

Next, we correlated IL-23 expression with plasma sCD14 (Fig. 4e) and LBP (Fig. 4f) levels at days 7 and 35 p.i. IL-23 expression significantly correlated with both sCD14 ($r = 0.5822$; $P = 0.0235$) and LBP ($r = 0.5860$; $P = 0.0226$) levels, suggesting that translocated bacterial products could play a role in activating peripheral blood monocytes during acute stages of infection. Interestingly, we observed a significantly high positive correlation ($r = 0.6783$; $P = 0.0077$) between plasma LBP levels and the mean fluorescence intensity (MFI) of CD14 expression on monocytes (Fig. 4g).

$CD8^+$ $Ki\text{-}67^+$ T cells are significantly increased during acute SIV infection and correlate with plasma viral loads but not sCD14 or LBP levels. Numerous studies have shown that translocation of microbial products is associated with systemic immune activation during HIV and SIV infections (6, 9). To determine if translocated microbial products play a role in acute immune activation, we evaluated the expression of Ki-67 on memory CD8 T cells at days 0, 7, 14, and 35 p.i. (Fig. 5a) and correlated that expression with sCD14 (Fig. 5b), LBP (Fig. 5c), and IL-17 (Fig. 5d) expression and plasma viral loads (Fig. 5e).

As previously reported (10–13), we observed a significant increase in $CD8^+$ $Ki\text{-}67^+$ T cells in peripheral blood during acute SIV infection. Surprisingly, we observed little or no correlation between $CD8^+$ $Ki\text{-}67^+$ T cells and sCD14 ($r = -0.1652$; $P = 0.4744$), LBP ($r = 0.1704$; $P = 0.2496$), or IL-17 ($r = -0.3117$; $P = 0.1620$) expression. In contrast, there was a significantly high positive correlation between $CD8^+$ $Ki\text{-}67^+$ T cells and plasma viral loads ($r = 0.6798$; $P = 0.0010$), suggesting that acute viral replication rather than translocated microbial products likely plays a primary role in driving acute immune activation.

DISCUSSION

Acute HIV and SIV infections are associated with significant perturbations in mucosal homeostasis that are characterized by a massive loss of CD4 T cells, viral replication, and damaged mucosal barrier (6, 25, 35–42). These changes occur within the first few

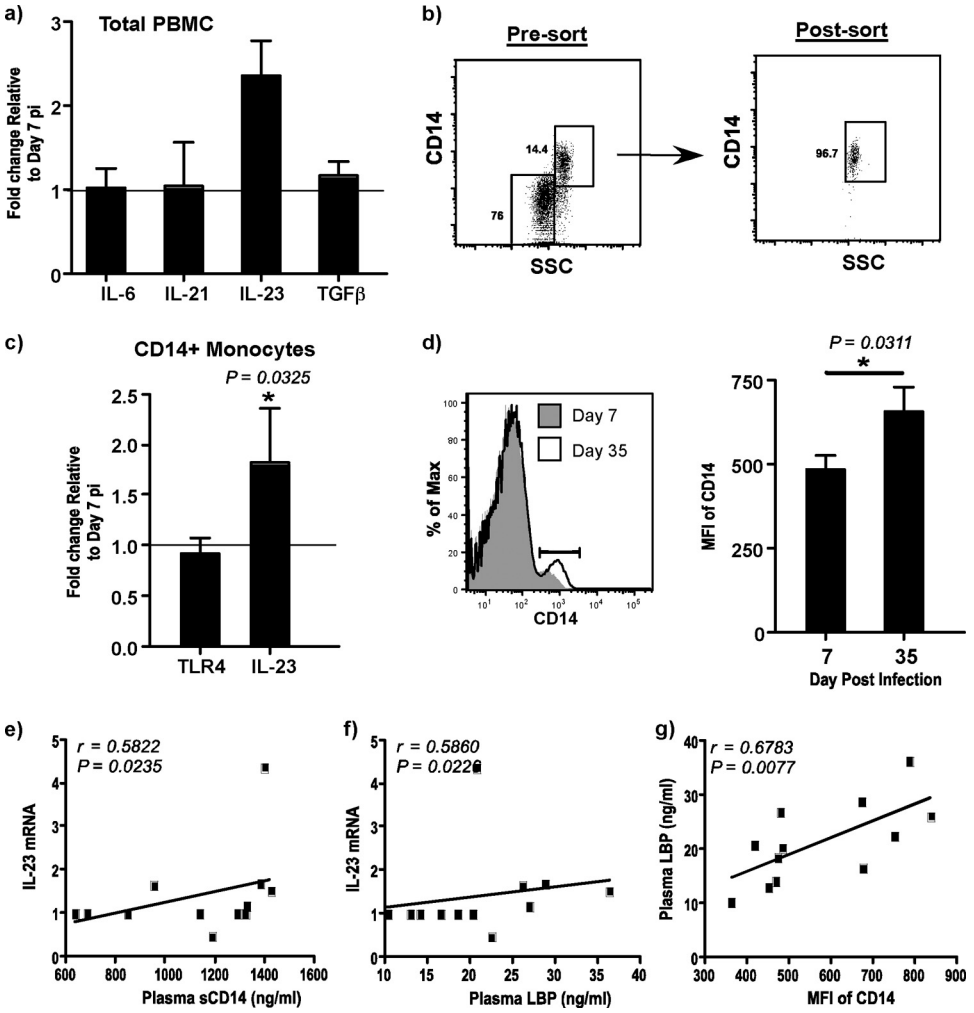

FIG 4 CD14$^+$ monocytes upregulate the density of CD14 expression and IL-23 mRNA levels during acute SIV infection. (a) *Ex vivo* expression of IL-6, IL-21, IL-23, and TGF-β in total PBMCs at day 7 and day 35 p.i. The fold change relative to the level at day 7 p.i. is shown. The line indicates day 7 p.i. as the baseline. (b) Gating strategy used to sort purified populations of CD14$^+$ monocytes by fluorescence-activated cell sorting. (c) *Ex vivo* expression of TLR4 and IL-23 expression in purified populations of CD14$^+$ monocytes at days 7 and 35 p.i. The fold change relative to the level at day 7 p.i. is shown. The line indicates day 7 p.i. as the baseline. (d) Histogram showing the density of CD14 expression from a representative animal at days 7 and 35 p.i. and data showing the MFI of CD14 expression on CD14$^+$ monocytes at days 7 and 35 p.i. (e to g) The correlations between the plasma levels of sCD14 and IL-23 expression on CD14$^+$ monocytes (e), plasma levels of LBP and IL-23 expression on CD14$^+$ monocytes (f), and plasma levels of LBP and MFI of CD14 expression on CD14$^+$ monocytes (g) at days 7 and 35 p.i. are shown.

weeks of infection and lead to a chronic state of inflammation that persists throughout the course of infection. Our findings suggest that systemic translocation of microbial products likely occurs very early during the course of infection.

Elevated sCD14 and LBP levels significantly correlated with the suppression of IL-17 production in CD4 T cells, supporting earlier observations that the loss of Th17 cells is associated with the translocation of microbial products (5, 43). Brenchley et al. (8) showed that peripheral blood CD4 T cells are skewed toward a Th1 phenotype, as opposed to a Th17 phenotype, during chronic HIV infection, whereas others have reported a loss of IL-17-producing CD4 T cells in peripheral blood during SIV infection (4, 16, 19, 44).

The suppression of IL-17 expression was not likely due to the paucity in IL-17-promoting cytokines, such as IL-6, IL-21, IL-23, and TGF-β, as we did not observe a suppression of these responses in PBMCs. Other studies have shown that plasma levels of IL-6, IL-21, and TGF-β were elevated during HIV and SIV infections (45–49), though studies have reported a decrease in IL-21 levels in the plasma (50) likely due to the loss of IL-21-producing Th17 cells (44). On the other hand, Zhao et al. showed that circulating IL-21 levels were increased during simian-human immunodeficiency virus infection (51). The relatively normal expression of IL-21 that we observed in total PBMCs may be due to the presence of CD8 T cells that were capable of producing IL-21, as some studies have reported (52).

Though altered cytokine levels may play a role in suppressing the induction of Th17 cells, the significantly low level of IL-17 expression at day 35 p.i. relative to that at day 7 p.i. after short-term stimulation with rIL-6 pointed to a role for other mechanisms in suppressing Th17 responses. In fact, we observed a significantly high negative correlation between IL-17 expression and

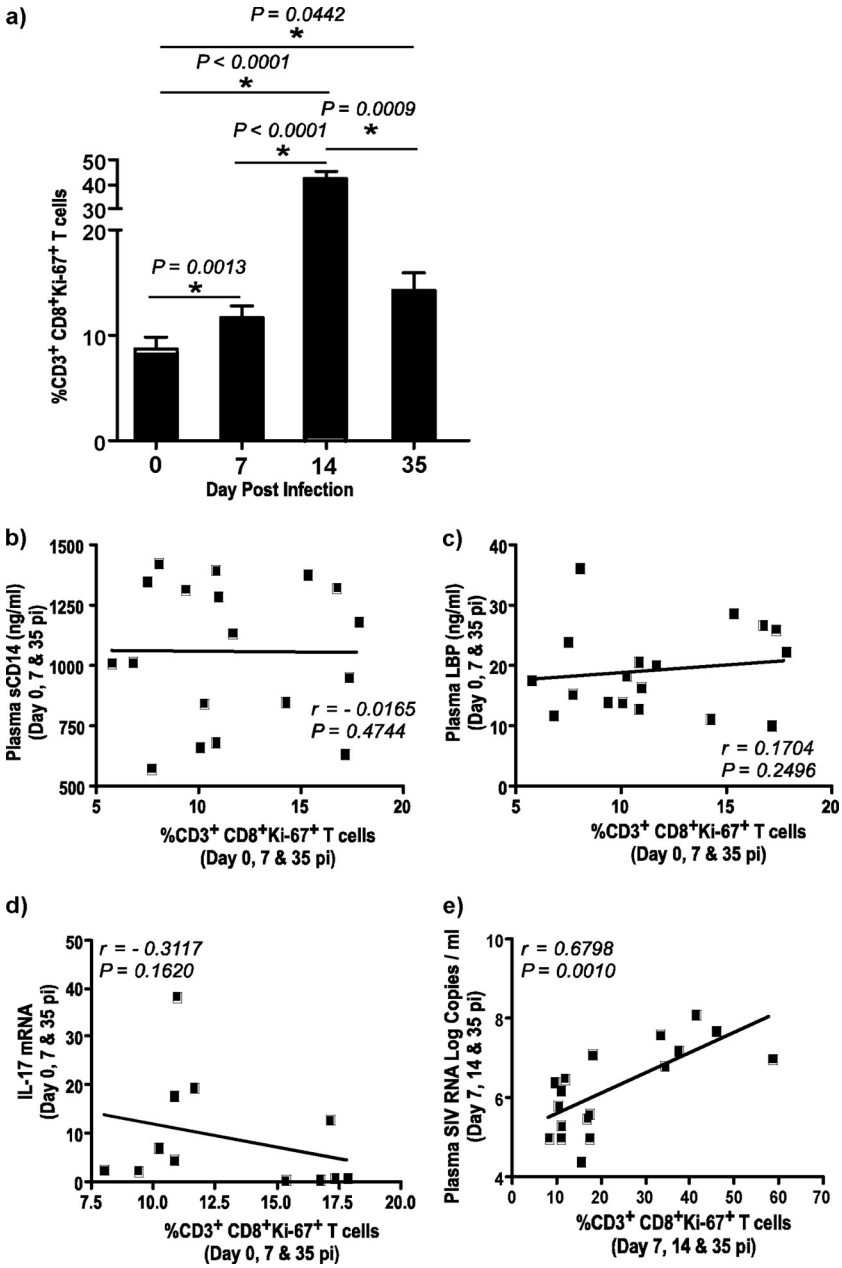

FIG 5 $CD3^+$ $CD8^+$ $Ki\text{-}67^+$ memory T cells significantly correlate with plasma viral loads but not sCD14 or LBP levels in plasma. (a) Percentage of $CD3^+$ $CD8^+$ $Ki\text{-}67^+$ memory T cells in peripheral blood. (b to e) The correlations between the percentage of $CD3^+$ $CD8^+$ $Ki\text{-}67^+$ memory T cells in peripheral blood and plasma sCD14 levels (b), plasma LBP levels (c), IL-17 expression in CD4 T cells (d), and plasma viral loads (e) are shown.

the negative regulators of IL-17 production in CD4 T cells, namely, PIAS3, SHP2, and SOCS3.

PIAS3 is a potent inhibitor of activated STAT3 signaling (53). PIAS3 transcripts are absent in Th17 cells but are present Th1 and Th2 cells, and a downregulation of PIAS3 was found to induce Th17 cells and increase the severity of experimental autoimmune encephalomyelitis (EAE) in mice (54). On the other hand, SHP2 is a tyrosine phosphatase that has been shown to interfere with the STAT3 signaling pathway, and the transduction of SHP2 was accompanied by a failure to induce Th17 cells (55). Likewise, SOCS3 has been shown to inhibit cytokine-induced phosphorylation of STAT3 and IL-17 production (29). Miller et al. (56) showed that SOCS3 mRNA levels in CD4 T cells were higher in HIV-infected patients than healthy subjects, and the overexpression of SOCS3 was found to inhibit the JAK/STAT pathway. Others have shown that IL-6 stimulation induces SOCS3 expression via the activation of the STAT3 signaling pathway (57). Taken together these observations suggest that the elevated levels of PIAS3, SHP2, and SOCS3 could potentially play a role in suppressing IL-17 expression during acute SIV infection.

Given that IL-6-mediated signaling requires the activation of STAT3, it was surprising that STAT3 mRNA was not upregulated following short-term stimulation with rIL-6 for 15 min. However, IL-6 primarily acts at the level of STAT3 activation and phosphorylation, and it is highly likely that longer periods (1 to 3 h) of

stimulation are required for IL-6-mediated expression of STAT3 mRNA, as some studies have suggested (58, 59).

It is not clear from our studies if protein levels for PIAS3, SHP2, and SOCS3 are increased in CD4 T cells, as we were unable to perform these studies for the lack of samples. The upregulated expression of the PIAS3, SHP2, and SOCS3 genes, however, along with the suppression of IL-17 expression, suggests that these negative regulatory factors may contribute to the suppression of Th17 cells very early during the course of infection.

It is highly likely that the suppression of IL-17 expression is due to the SIV-mediated loss of Th17 cells, as has been reported previously (8, 19, 60), or due to other mechanisms, such as those mediated by the induction of indoleamine 2,3-dioxygenase-1 (61, 62) or the paucity of IL-21-producing CD4 T cells (44). Our studies do not rule out a role for these mechanisms but suggest that additional mechanisms, such as those that negatively regulate the IL-17 signaling pathway, may play a role in suppressing the expression of Th17 cells during SIV infection and that these suppressive mechanisms were apparent very early during the course of infection.

Increased translocation of microbial products correlated with IL-23 expression in monocytes and was associated with a significant upregulation in the density of CD14 expression, suggesting that translocated products could contribute to the activation of monocytes during acute infection. Ancuta et al. (34) demonstrated that microbial translocation was associated with increased monocyte activation in HIV-infected patients, whereas Manuzak et al. (63) showed that bacterial antigens increase IL-23 production by peripheral blood monocytes. Louis et al. (64) showed that monocytes from patients with primary HIV infection significantly enhance IL-23 production in response to LPS stimulation. Kader et al. (16) showed that IL-23 mRNA levels were upregulated in the mucosa during acute stages of SIV infection and stayed high even after the loss of CD4 T cells.

Interestingly, neither the suppression of IL-17 expression nor increased levels of sCD14 or LBP directly correlated with acute immune activation, suggesting that mechanisms other than translocated microbial products likely drive the immune activation observed very early during infection. Acute infection is characterized by a massive amplification of viral infection that is accompanied by immune activation during the early stages of infection (10–13), likely due to the release of various proinflammatory mediators (65). In line with this argument, we observed a significantly high positive correlation between plasma viral loads and $CD8^+$ $Ki\text{-}67^+$ T cells in peripheral blood. Interestingly, immune activation appeared to precede elevated levels of plasma sCD14 and LBP; $CD8^+$ $Ki\text{-}67^+$ T cell levels were significantly higher at day 7 p.i. than at day 0, without an apparent change in sCD14 and LBP levels.

Though sCD14 and LBP levels did not correlate with acute immune activation, they significantly correlated with IL-23 expression in monocytes and the MFI of CD14 expression on monocytes, suggesting that translocated products could potentially interact with the higher levels of CD14 on monocytes and contribute to the generalized state of inflammation during acute SIV infection. The levels of sCD14 and LPB that we observed during acute stages of infection were lower than what has been reported in chronically infected animals (2) and in line with those described in previous reports showing low but detectable levels of LPS in the mucosa during early stages of SIV infection (6). Given the low levels of translocated products during early infection, it is highly likely that the direct effect of translocated microbial products on acute immune activation is probably masked by the release of inflammatory mediators associated with massive viral replication. Breed et al. (66) showed that rhesus macaques infected with the SIVmac239ΔGY mutant strain exhibited wild-type acute viremia and immune activation but maintained their mucosal CD4 T cells and displayed a lack of microbial translocation.

In conclusion, our studies show that acute SIV infection is characterized by a significant increase in markers of microbial translocation that correlate with suppressed Th17 responses. Elevated expression of various negative regulatory genes likely plays a role in suppressing IL-17 expression in CD4 T cells during acute stages of SIV infection. Active viral replication rather than translocated microbial products, however, correlated with Ki-67 expression on CD8 T cells, suggesting a primary role for viral replication in acute immune activation.

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