

Persistent Microbial Translocation and Immune Activation in HIV-1–Infected South Africans Receiving Combination Antiretroviral Therapy

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Background. Microbial translocation contributes to immune activation and disease progression during chronic human immunodeficiency virus type 1 (HIV-1) infection. However, its role in the African AIDS epidemic remains controversial. Here, we investigated the relationship between markers of monocyte activation, plasma lipopolysaccharide (LPS), and HIV-1 RNA in South Africans prioritized to receive combination antiretroviral therapy (cART).

Methods. Ten HIV-1–negative African controls and 80 HIV-1–infected patients with CD4 T cell counts <200 cells/ μ L were sampled prior to ($n = 60$) or during ($n = 20$) receipt of effective cART. Viral load was measured by Nuclisens; LPS by the Limulus amoebocyte lysate assay; monocyte and T cell subsets by flow cytometry; and soluble CD14, cytokines, and chemokines by enzyme-linked immunosorbent assay and customized Bio-Plex plates.

Results. Three distinct sets of markers were identified. CCL2, CXCL10, and CD14⁺CD16⁺ monocyte levels were positively correlated with HIV-1 viremia. This finding, together with cART-induced normalization of these markers, suggests that their upregulation was driven by HIV-1. Plasma interleukin-6 was associated with the presence of opportunistic coinfections. Soluble CD14 and tumor necrosis factor were linked to plasma LPS levels and, as observed for LPS, remained elevated in patients receiving effective cART.

Conclusions. Microbial translocation is a major force driving chronic inflammation in HIV-infected Africans receiving cART. Prevention of monocyte activation may be especially effective at enhancing therapeutic outcomes.

Excessive immune activation is a major force driving human immunodeficiency virus type 1 (HIV-1) replication and progression to AIDS [1–5]. In most patients, combination antiretroviral therapy (cART) results in a sustained reduction in plasma HIV-1 RNA and decreased AIDS-related morbidity and mortality [6]. However, cART does not always lead to a normalization

of systemic inflammation [7–9]. In ~20% of patients, persistent inflammation is associated with poor CD4 T cell recovery, suboptimal therapeutic gains, and an increased risk of cancer and other non-AIDS-related diseases, especially in patients who begin therapy after their CD4 T counts decrease to <200 cells/ μ L [9–13].

Cells of the monocyte/macrophage lineage play a key role in the orchestration of innate and adaptive responses and in the initiation and resolution of inflammation [14]. They respond to a wide range of antigenic and microbial stimuli and the outcome of these interactions depends on the cell's differentiation and polarization status [15]. If properly regulated, these responses result in activation of effector mechanisms and killing of invading pathogens. If excessive or inappropriate, they lead to immune dysregulation, coinfections, uncontrolled inflammation, sepsis, and death [16–18].

Studies conducted primarily in North America sug-

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Table 1. Demographic Characteristics of Human Immunodeficiency Virus Type 1 (HIV-1)-Infected Patients

Characteristic	HIV-1-infected patients		
	Without opportunistic infection	With opportunistic infection	Received cART for >1 year
Sex			
Male	11	6	8
Female	29	14	12
Age, mean years \pm SD	38.5 \pm 11.1	35.4 \pm 8.6	39.3 \pm 10.7
CD4 T cell count, mean cells/ μ L \pm SD	138 \pm 117	133 \pm 148	386 \pm 208
Plasma HIV-1 load, mean log copies/mL \pm SD	4.31 \pm 0.70	4.53 \pm 0.88	<LDL
Opportunistic infection			
HSV-2	...	1	...
Tuberculosis	...	3	...
<i>Candida albicans</i>	...	10	...
Other bacterial infections	...	6	...

NOTE. Data are no of patients, unless otherwise indicated. All 80 patients had heterosexually acquired HIV-1 infection, and all were infected with HIV-1 subtype C. cART, combination antiretroviral therapy; HSV-2, herpes simplex virus 2; LDL, lower detection limit; SD, standard deviation.

gest that increased levels of circulating lipopolysaccharide (LPS) and other bacterial products, a consequence of microbial translocation across a damaged intestinal mucosal, can contribute to aberrant immune activation in HIV-1-infected individuals [8, 19–24]. Interactions between LPS and the CD14–Toll-like receptor 4 (TLR4) complex on monocytes and tissue macrophages are associated with increased secretion of cytokines that drive T cell activation and cause activation induced cell death [20]. Although microbial translocation plays an important role in HIV-1 pathogenesis in the developed world, it is unclear whether it contributes to HIV-1 disease in Africa. A recent study in Uganda found no association between circulating LPS and progression to AIDS [25]. In contrast, a study of female sex workers in Kenya detected significant associations between HIV-1 infection, plasma LPS, and TLR4 messenger RNA (mRNA) expression levels in peripheral blood mononuclear cells [26]. Inflammatory responses to LPS were highly variable among individuals, suggesting that differences in host immunity and background levels of immune activation can modify the impact of microbial translocation on HIV-1 disease [26].

In this study, we examined the interplay between monocyte activation, HIV-1 viremia, and circulating levels of LPS in HIV-1-infected South Africans with low CD4⁺ T cell counts (<200 cells/ μ L), both before and after 1 year of successful cART. Increased plasma CCL2 and CXL10 levels and an increased frequency of proinflammatory (CD14⁺CD16⁺) monocytes were positively correlated with high levels of HIV-1 viremia. This finding, together with normalization of these markers during receipt of cART, suggests that their up-regulation was driven primarily by HIV-1. Soluble CD14 (sCD14) and tumor necrosis factor (TNF) levels correlated with LPS levels (before and dur-

ing cART, respectively) and remained persistently elevated even after >1 year of successful therapy, suggesting that, as observed in North America, microbial translocation is a major driver of chronic inflammation in HIV-1-infected South Africans receiving cART.

METHODS

Study cohorts. HIV-1-infected individuals ($n = 80$) were recruited from the Comprehensive Care, Management, and Treatment Clinic of the Tshwane District Hospital in Pretoria. HIV-1-negative volunteers were recruited from an outpatient clinic at Tshwane District Hospital ($n = 10$). cART-naive patients were sampled at the time of presentation; treated patients were sampled during scheduled clinical visits. All participants gave written informed consent. Three distinct cohorts with stage III or IV disease (World Health Organization criteria) were examined: cART-naive patients with ($n = 20$) and without ($n = 40$) opportunistic coinfections, and cART-treated patients with undetectable plasma viremia of >6 months duration ($n = 20$). Patients were considered to be free of opportunistic coinfections if they had negative results for tuberculosis in culture and had no clinical evidence of opportunistic disease. Patients with opportunistic coinfections had a range of bacterial, viral, and fungal coinfections typical of the population (Table 1). CD4⁺ T counts, plasma HIV-1 RNA levels, full blood counts, alanine aminotransferase levels, and aspartate aminotransferase levels were obtained for cART-naive patients as part of their routine care; CD4⁺ T cell counts and viral loads were measured for all cART-treated patients every 6 months. Treatment consisted of 30 mg of stavudine and 150 mg lamivudine twice daily

and either 600 mg of efavirenz once daily or nevirapine at a post-initiation dose of 200 mg twice daily. The Ethics Committee of the University of Pretoria approved the study.

Plasma viremia. EDTA plasma was stored in 1.0-mL aliquots at -80°C until needed for RNA isolation. RNA was extracted using the Magnetic Extraction Reagent kit from BioMerieux. HIV-1 RNA quantifications were performed with 1.0 mL of plasma with use of the Nuclisens Easy Q HIV-1 v.1.2 kit, an assay with a lower detection limit of 40–50 copies/mL.

CD4⁺ T cell counts, CD8⁺ T cell activation, and inflammatory monocyte populations. CD4⁺ T cell counts and the percentage of total T lymphocytes were evaluated using a cocktail of anti-human murine CD45 (clone J.33) and CD4 (clone 13B8.2) monoclonal antibodies (Beckman Coulter). The frequency of activated CD8⁺ T cells was determined by measuring the expression of CD38 (clone NIMR-5) and HLA-DR (clone B8.12.2). Inflammatory monocytes were identified using CD14 (clone RM052) and CD16 (clone 3GB) monoclonal antibodies. Samples were vortexed, were incubated in the dark for 10 min at room temperature, and were placed in TQ-Prep (Beckman Coulter) to lyse red cells and fix leukocytes. Absolute counts and percentages were determined using an FC500 flow cytometer (Beckman Coulter). Proinflammatory monocytes, identified based on forward and side scatter properties, were defined as the percentage of total monocytes that coexpressed CD14 and CD16.

Quantification of soluble markers. Plasma LPS levels were quantified by the Limulus Amoebocyte Lysate assay QCL-1000 (Lonza). Samples were diluted 1:3 with Limulus Amoebocyte Lysate Reagent water, were inactivated for 30 min at 65°C , and were tested in 96-well microplates [21]. sCD14 was measured by enzyme-linked immunosorbent assay (R&D Systems). Cytokine and chemokine levels were quantified using customized Bio-Plex plates (Bio-Rad). Samples were diluted 1:5 with Human Serum Diluent (Bio-Rad) prior to quantification. All assays were performed according to manufacturer's instructions.

Statistical analysis. Results are reported as mean values \pm standard deviation (SD). Analysis of variance was determined using the Mann-Whitney *U* test. Spearman correlations (*r* and *P* values) were calculated to determine the relationships between HIV viremia, plasma LPS levels, and activation markers.

RESULTS

Study participants. The cohort included 60 cART-naive HIV-1-infected individuals with and without opportunistic coinfections, 20 cART-experienced HIV-1-infected individuals treated with 2 nucleoside reverse-transcriptase inhibitors and 1 nonnucleoside reverse-transcriptase inhibitor with suppressed viral replication, and 10 healthy HIV-1-negative African controls. No differences were observed in the demographic characteristics between cART-naive and cART-treated patients or

in the mean CD4 T cell counts between the 2 cART-naive groups. cART-naive patients without ($n = 40$) and with ($n = 20$) opportunistic coinfections had a mean age (\pm SD) of 38.5 ± 1.1 and 35.4 ± 8.6 years and a mean CD4 T cell count (\pm SD) of 138 ± 117 and 133 ± 148 cells/ μL , respectively (Table 1). All cART-naive patients presented with CD4 T cell counts <200 cells/ μL and, thus, were eligible for therapy according to South African treatment guidelines. Patients receiving successful cART for >1 year ($n = 20$) had a mean age (\pm SD) of 39.3 ± 10.7 years and a higher mean CD4⁺ T cell count (386 ± 208 cells/ μL) than did untreated patients with ($P < .001$) and without ($P < .001$) opportunistic coinfections (Table 1). The ratios of male:female patients in the cART-naive, treated, and uninfected groups were 0.40, 0.66, and 0.57, respectively.

HIV-1 replication and acute opportunistic coinfections are associated with increased plasma LPS. LPS levels (\pm SD) were significantly higher among HIV-1-infected patients relative to uninfected African controls (2.14 ± 0.57 vs 1.10 ± 0.26 EU/mL; $P < .001$). LPS concentrations (\pm SD) were also higher among HIV-1-infected cART-naive patients with opportunistic coinfections, compared with those who did not have an overt clinical opportunistic coinfection (2.42 ± 0.34 vs 2.030 ± 0.43 EU/mL; $P < .01$) (Figure 1A). Increased plasma LPS concentrations in patients with opportunistic coinfections suggests that, in addition to the translocation of commensal bacteria, secondary coinfections can also contribute to circulating levels of LPS. Although cART was associated with a partial reduction in LPS levels (\pm SD) (2.16 ± 0.43 vs 1.66 ± 0.32 EU/mL; $P < .001$), plasma levels remained well above control values even after 1 year of effective therapy (1.66 ± 0.32 vs 1.10 ± 0.25 EU/mL; $P < .001$), possibly because of a delay in intestinal repair or residual low-level viral replication and sustained microbial translocation (Figure 1B) [27]. There were no statistically significant differences in HIV-1 RNA levels (\pm SD) among infected patients with and without opportunistic coinfections (4.31 ± 0.70 vs 4.53 ± 0.88 log copies/mL; $P = .112$) (Table 1). All 20 patients receiving cART for >1 year were good responders and had undetectable viremia (ie, <50 copies/mL) for >6 months prior to sampling.

HIV-1 replication is associated with increased levels of CCL2, CXCL10, and inflammatory monocytes. Plasma CCL2 and CXCL10 levels (\pm SD) and the frequency of circulating inflammatory (CD14⁺CD16⁺) monocytes (\pm SD) were all clearly elevated in HIV-1-infected patients, compared with uninfected controls (CCL2, 166.8 ± 80.61 vs 32.30 ± 14.83 pg/mL [$P < .001$]; CXCL10, 6491 ± 3810 vs 2230 ± 2890 pg/mL [$P < .001$]; CD14⁺CD16⁺, $26.38\% \pm 9.8\%$ vs $11.83\% \pm 3.33\%$ [$P < .001$]) (Figure 2A). Opportunistic coinfections were not associated with any additional increases. HIV-1 RNA levels in patients with and without opportunistic coinfections were pos-

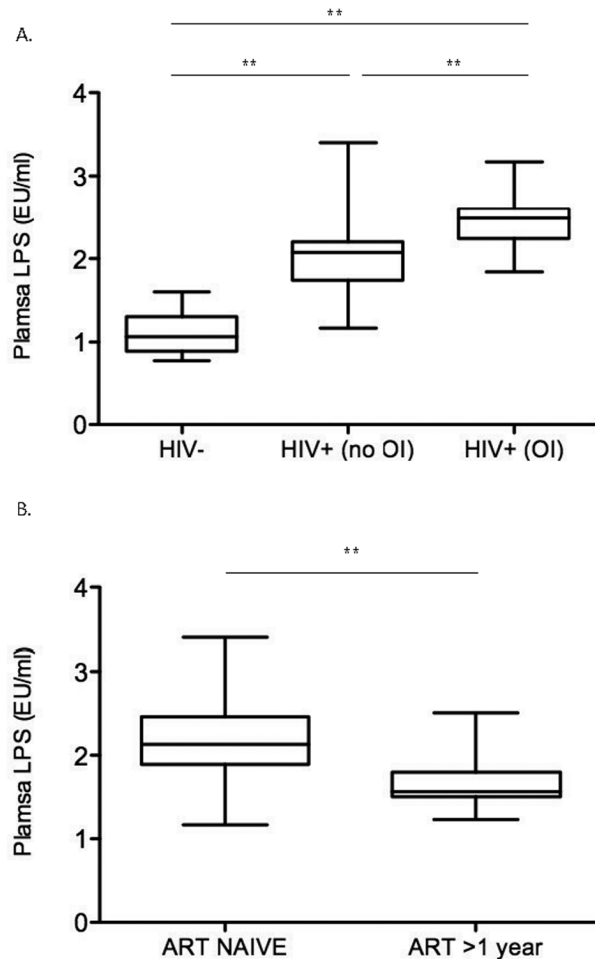


Figure 1. South African patients prioritized to receive combination antiretroviral therapy (cART) display increased levels of circulating lipopolysaccharide (LPS), both before and during successful cART. *A*, Levels of circulating LPS are significantly higher in human immunodeficiency virus type 1 (HIV-1)-infected patients, compared with healthy uninfected controls, and in HIV-1-infected patients with opportunistic coinfections (OIs), compared with those with no evidence of an OI. Plasma levels of LPS were measured in HIV-1-seronegative ($n = 10$) African controls and in HIV-1-infected cART-naive ($n = 40$) Africans with ($n = 20$) and without ($n = 40$) representative OIs. All HIV-1-infected patients had CD4 T cell counts <200 cells/ μ L at the time of presentation and, thus, according to South African treatment guidelines were eligible for cART. *B*, cART is only partially effective in reducing circulating LPS in Africans with chronic HIV-1 infection and low CD4 T cell counts. Plasma LPS levels were measured in cART-naive ($n = 60$) and cART-treated ($n = 20$) patients (>1 year after the initiation of a successful treatment with cART). Differences between the various groups were calculated using the Mann-Whitney test. $**P < .001$.

itively correlated with circulating concentrations of CCL2 and CXCL10 and the frequency of circulating CD14⁺CD16⁺ monocytes (Figure 2B).

The view that HIV-1 was directly responsible for the increase in CCL2, CXCL10, and CD14⁺CD16⁺ levels (\pm SD) was further

confirmed by the normalization of these activation markers to control values in cART-treated patients with undetectable plasma virus levels (CCL2, 43.17 ± 17.89 vs 32.30 ± 14.83 pg/mL [$P = .078$]; CXCL10, 2576 ± 1510 vs 2230 ± 2890 pg/mL [$P = .082$]; CD14⁺CD16⁺, $15.19\% \pm 3.93\%$ vs $11.83\% \pm 3.33\%$ [$P = .066$]) (Figure 2C). No correlations were detected between LPS (or CD4 count) and CCL2, CXCL10, or CD14⁺CD16⁺ in either cART-naive or treated patients.

Chronic HIV-1 infection, on its own, does not lead to a significant up-regulation of interleukin (IL)-6. Among other proinflammatory cytokines, increased levels of IL-6 have been associated with acute infections [28, 29]. IL-6 levels (\pm SD) were higher in HIV-1-infected patients with clinically detectable opportunistic coinfections (43.68 ± 36.92 pg/mL), compared with healthy uninfected controls (14.50 ± 10.15 pg/mL; $P < .05$) (Figure 3A). Although there was a trend toward increased IL-6 levels in HIV-1-infected patients without opportunistic coinfections, compared with controls, this relationship did not reach statistical significance ($P = .153$). IL-6 levels (\pm SD) in cART-treated patients without opportunistic coinfection were comparable to those in uninfected Africans (22.12 ± 15.60 and 14.50 ± 10.15 pg/mL, respectively) (Figure 3B). No correlations were detected between circulating IL-6 and plasma HIV-1 RNA or LPS, suggesting that the up-regulation of IL-6 was primarily driven by acute opportunistic infections.

sCD14 and TNF are increased in HIV-1-infected Africans and remain persistently elevated during cART. sCD14 and TNF levels (\pm SD) were markedly increased in HIV-1-infected cART-naive patients, relative to uninfected controls (sCD14, 2.47 ± 0.78 vs 1.61 ± 0.44 μ g/mL [$P = .007$]; TNF, 90.14 ± 92.52 vs 11.40 ± 8.79 pg/mL [$P < .001$]) (Figure 4A). Both markers are polyclonal in nature and are routinely up-regulated in response to a broad range of pathogens. The lack of a further increase in patients with opportunistic coinfections suggests that sCD14 and TNF may already be maximally produced in patients with late-stage HIV-1 disease. Of particular importance was the finding that sCD14 and TNF levels remained persistently elevated after 1 year of successful cART (Figure 4B).

Plasma levels of sCD14 and TNF are linked to LPS. In the absence of overt opportunistic coinfections, a positive correlation (Spearman correlation r , 0.48; $P = .002$) was observed between plasma LPS and sCD14 levels (Figure 5) in cART-naive patients. This observation suggests that, in untreated patients, the increase in sCD14 was driven predominantly by microbial translocation. Interestingly, in cART-naive patients with opportunistic coinfections, a negative rather than a positive correlation was detected between LPS and sCD14 (Spearman correlation r , -0.52 ; $P = .020$). Thus, the added burden of secondary coinfections appears to interfere (either directly or indirectly) with the complex relationships between gut-associated microbial translocation and sCD14. No correlations

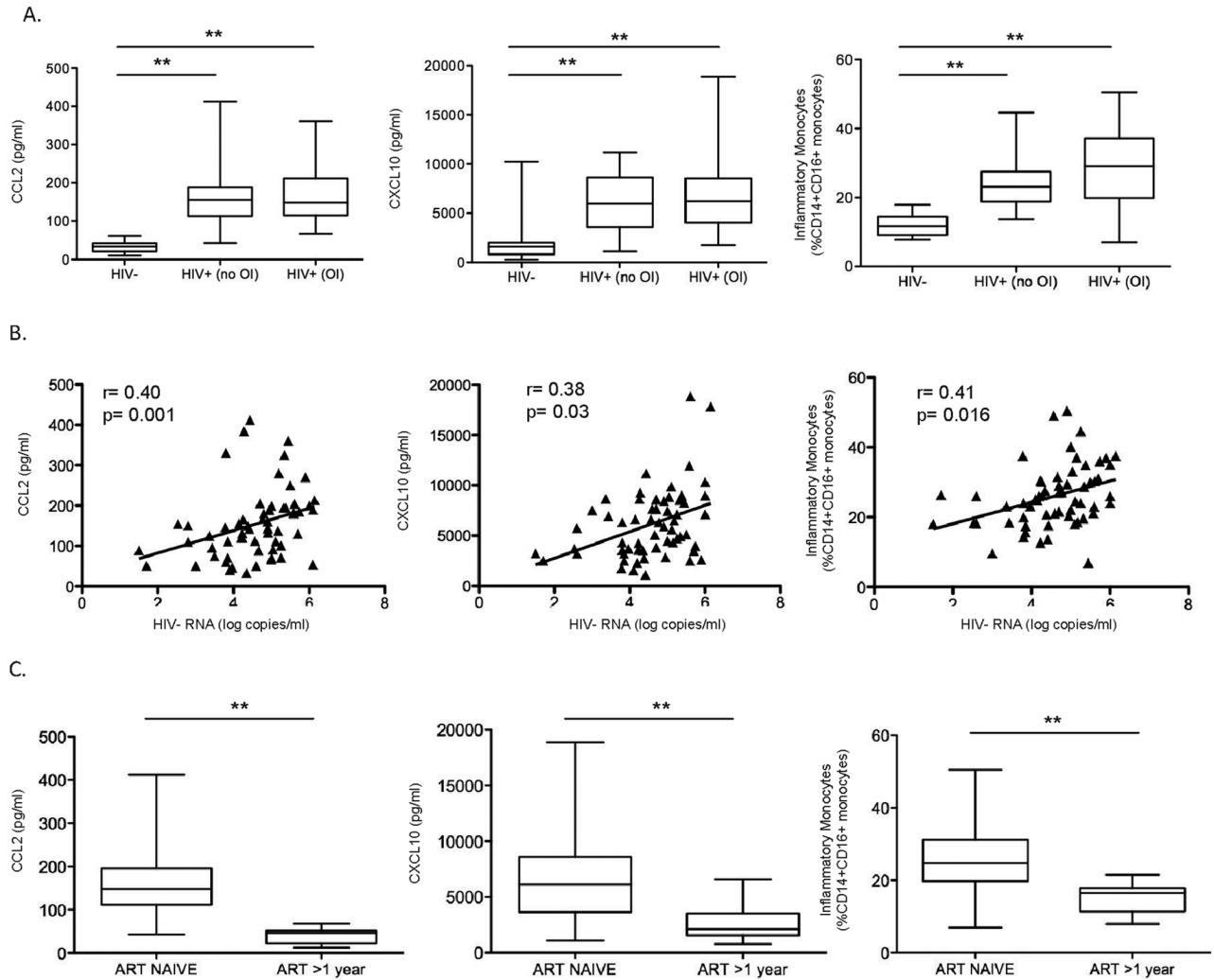


Figure 2. Increased levels of CCL2, CXCL10, and CD16 are linked to human immunodeficiency virus type 1 (HIV-1) replication. *A*, Untreated chronic HIV-1 infection is associated with a marked increase in CCL2 and CXCL10 and in the frequency of monocytes expressing CD16. Plasma levels of CCL2 and CXCL10 were quantified in chronically infected, ART-naive patients with ($n = 40$) and without ($n = 20$) opportunistic coinfections (OIs) and in uninfected African controls ($n = 10$). The frequency of circulating CD14⁺CD16⁺ monocytes was determined by flow cytometry. Monocytes were distinguished from natural killer cells and granulocytes on the basis of their forward and side scatter characteristics and their coexpression of CD14. Differences in CCL2, CXCL10, and inflammatory monocytes among patient groups were examined using the Mann-Whitney test. ** $P < .001$. *B*, CCL2, CXCL10, and CD14⁺CD16⁺ monocyte levels are positively correlated with HIV-1 loads. Spearman correlations (r and P values) were used to examine the relationships between each activation marker (CCL2, CXCL10, and the percentage of CD14⁺CD16⁺ monocytes) and plasma levels of HIV-1 RNA. *C*, Successful combination antiretroviral therapy reduces CCL2 and CXCL10 production and the frequency of inflammatory monocytes to levels observed in HIV-1-negative individuals. HIV-1-infected patients with undetectable viral loads (for >6 months) who had been treated with 2 nucleoside reverse-transcriptase inhibitors and 1 nonnucleoside reverse-transcriptase inhibitor for at least 1 year (mean duration \pm SD, 14 ± 2.2 months) were defined as good responders. P values were calculated using the Mann-Whitney test. * $P < .05$; ** $P < .001$.

were detected between sCD14 and circulating LPS levels in cART-treated patients, presumably because of complex changes occurring in the immune environment during treatment.

Interestingly, no correlation was observed between TNF levels and plasma LPS in cART-naive patients (Figure 5), suggesting that, in the absence of treatment, TNF secretion is driven by a combination of viral and microbial factors. However, a direct positive correlation was observed between LPS

and circulating levels of TNF (Spearman correlation r , 0.52, $P = .019$) in patients receiving cART, supporting the view that microbial translocation is an important contributor to persistent inflammation in patients receiving cART.

Interferon (IFN)- γ and CD8 T cell activation are not directly linked to plasma viremia, microbial translocation, or monocyte activation. Because monocyte activation can affect the activation status of T cells, we also looked for potential

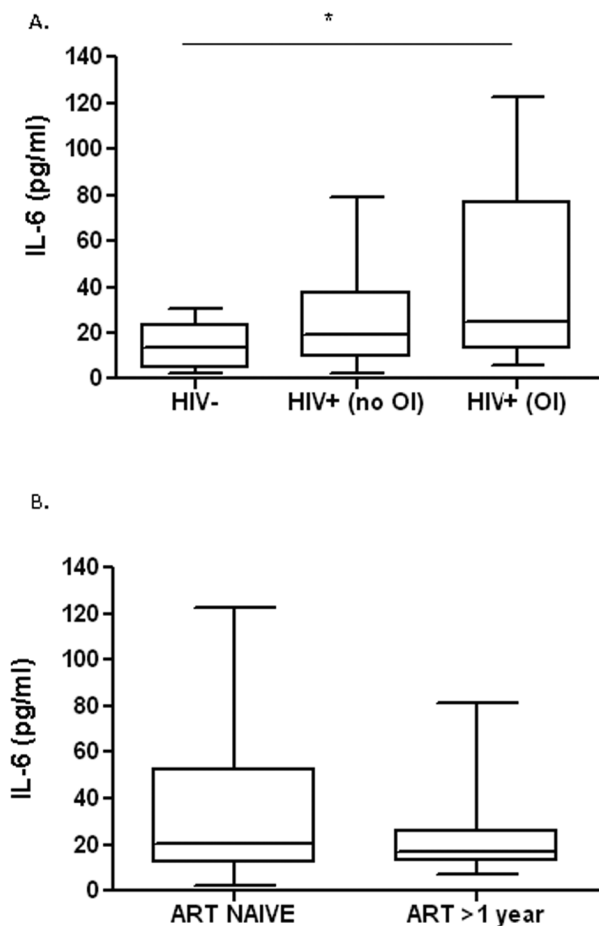


Figure 3. Increased interleukin (IL)-6 is associated with the presence of acute opportunistic disease. *A*, Circulating levels of IL-6 were higher in HIV-1-infected patients with opportunistic coinfections (OI), compared with uninfected African controls. IL-6 levels in plasma were quantified by enzyme-linked immunosorbent assay in HIV-1-seronegative African controls ($n = 10$) and in HIV-1-infected cART-naive Africans with ($n = 20$) and without ($n = 40$) representative OIs. *B*, IL-6 levels in patients receiving combination antiretroviral therapy (cART) were similar to those of untreated patients without OIs. IL-6 levels were measured in cART-naive ($n = 60$) and cART-treated ($n = 20$) patients (>1 year after initiation of successful treatment with 2 nucleoside reverse-transcriptase inhibitors and 1 nonnucleoside reverse-transcriptase inhibitor). Significant differences and P values were determined using the Mann-Whitney test. * $P < .05$.

associations between markers of monocyte activation, LPS, HIV-1 load, IFN- γ production, and the frequency of activated CD8⁺ T cells. IFN- γ and HLA-DR⁺CD38⁺ CD8⁺ T cell levels (\pm SD) were significantly higher in HIV-1-infected individuals, compared with uninfected controls (IFN- γ , 570.3 \pm 548.1 vs 83.10 \pm 33.95 pg/mL; HLA-DR⁺CD38⁺CD8⁺ T cells, 42.48% \pm 13.08% vs 9.4% \pm 3.32%) (Figures 6). No correlations were found between IFN- γ or HLA-DR⁺CD38⁺CD8⁺ T

cell levels and HIV-1 viremia, plasma LPS, or markers of monocyte activation, emphasizing the complexity of T cell activation during late-stage disease. IFN- γ levels remained persistently elevated after 1 year of effective cART. The sustained up-regulation of IFN- γ during cART is likely to be complex and involve multiple factors related to low-level viral replication and sustained microbial translocation. In contrast, cART was associated with a marked decrease in the HLA-DR and CD38 expression on CD8 T cells (Figure 6), suggesting that HIV-1 plays a fundamental role, either directly or indirectly, in the activation of CD8 T cells.

DISCUSSION

HIV-1 infection is associated with systemic immune activation and cytokine/chemokine dysregulation, all of which contribute to HIV-1 pathogenesis [1–5]. Recent studies also suggest that cART is only partially effective at controlling chronic immune activation and that persistent activation during treatment is likely to foster suboptimal CD4 T cell responses and therapeutic failure [7–9, 22]. This is of particular concern in South Africa, where patients are prioritized for treatment only when their CD4 T cells decrease to <200 cells/ μ L. Such persons are more likely to exhibit persistent inflammation, suboptimal CD4 T cell gains, and an increased risk of developing non-AIDS-related diseases [9]. For this reason, we investigated the factors driving immune activation in Africans with low CD4 T cell counts (<200 cells/ μ L) who had been prioritized to receive cART. We found that monocyte activation was complex and differentially driven by multiple factors, including HIV-1, microbial translocation (subclinical endotoxemia), and acute opportunistic coinfections. Some markers correlated with high levels of viral replication, whereas others were more closely linked to LPS levels and coinfections. Furthermore, not all aspects of monocyte (and T cell) activation were responsive to cART. Of particular note was the finding that, in patients receiving cART, TNF levels remained persistently elevated and correlated with plasma LPS, despite the effective clearance of HIV-1 RNA to undetectable levels.

Although microbial translocation is an important driver of HIV-1 pathogenesis in North America [19–21], its contribution to HIV-1 disease in Africa, a continent where enteric pathogens and opportunistic coinfections are endemic, remains controversial. Our data demonstrating increased levels of circulating LPS in HIV-1-infected South Africans, compared with uninfected African controls, are consistent with data from North America and Kenya [19–21, 26] but differs from a recent longitudinal study from Uganda [25]. In the latter study, the first of its kind, the investigators did not detect any significant correlations between circulating levels of LPS, inflammatory cytokines, and HIV-1 disease progression (defined as a decrease

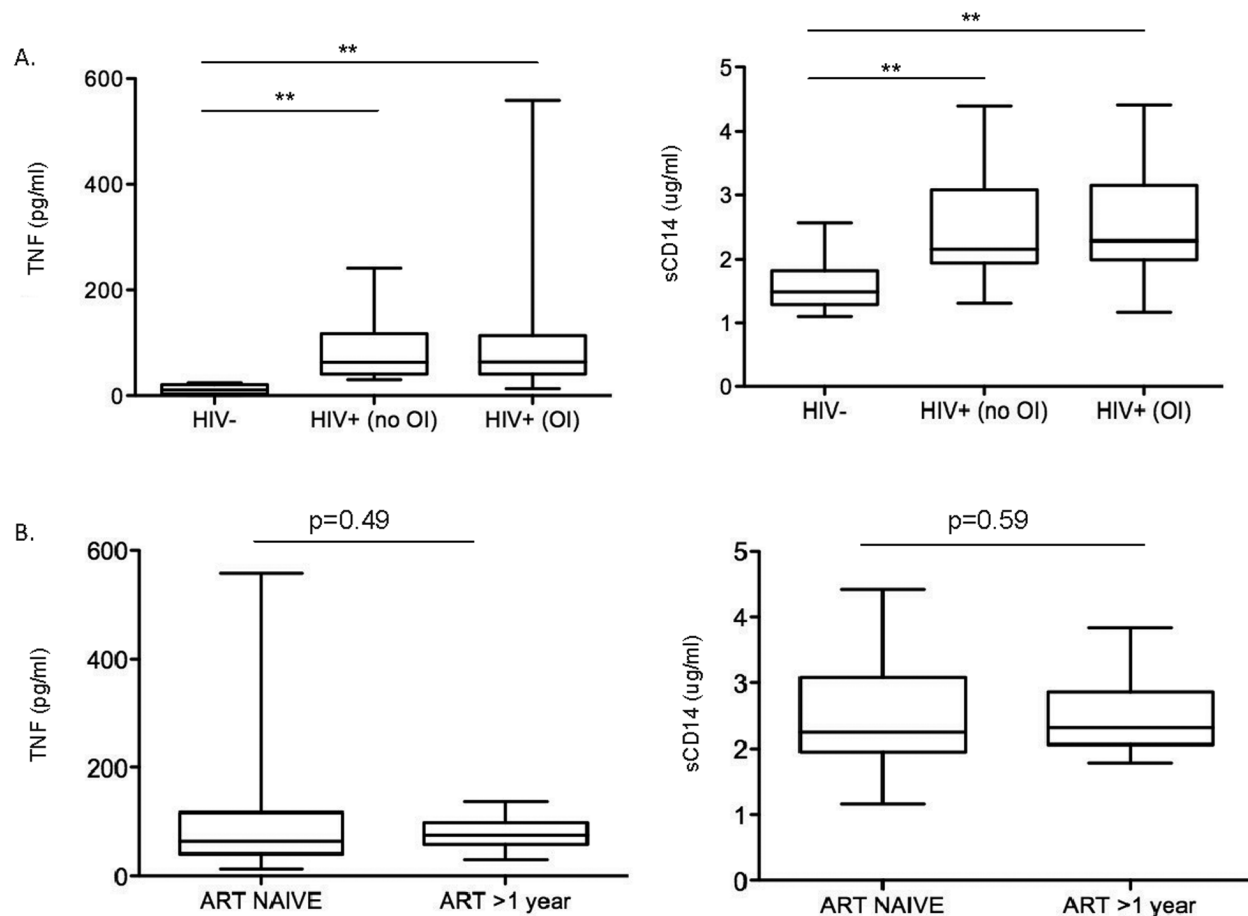


Figure 4. Tumor necrosis factor (TNF) and soluble CD14 (sCD14) levels remain up-regulated despite >1 year of successful combination antiretroviral therapy (cART). *A*, Chronic human immunodeficiency virus type 1 (HIV-1) infection is associated with a marked up-regulation of plasma TNF and sCD14. Levels of TNF and sCD14 were quantified in the plasma of HIV-1-infected cART-naive Africans with and without opportunistic coinfections and in uninfected controls. *B*, cART is ineffective at lowering TNF and sCD14 levels in African patients initiating treatment with low CD4 counts. TNF and sCD14 levels in cART-naive patients ($n = 60$) were compared with those in treated patients with undetectable (<50 copies/mL for >6 months) plasma HIV-1 RNA ($n = 20$). Significant differences and P values were determined using the Mann-Whitney test. ** $P < .001$.

in CD4 T cell counts or time to death) and concluded that microbial translocation was not an important contributor to HIV-1 disease in Africa [25]. The reason for the apparent discrepancy between studies is not known but may be related to the complexity of the activation process and the specific subset of immune markers that were evaluated. As suggested by Lester et al [26], it may be the nature of the host response to LPS rather than the amount of LPS that determines disease progression. Lester et al have reported that LPS-induced signaling through the TLR4/CD14 receptor complex is typically associated with a down-regulation of TLR4 mRNA and a decreased responsiveness to subsequent LPS stimulations [26], a phenomenon commonly described as endotoxin tolerance [30]. In contrast, TLR8-mediated signaling, induced by pre-exposure to HIV-1 RNA, is associated with an up-regulation of TLR4 mRNA, enhanced responsiveness to LPS stimulation, and a loss

of the tolerance [26]. Given this scenario, high levels of viral replication, especially during the late stage of chronic HIV-1 infection, would be expected to drive sustained LPS responses and continued immune activation, facilitating further disease progression.

Interestingly, in our study, sCD14 secretion was positively correlated with LPS levels in patients without opportunistic coinfections and was negatively correlated in patients with opportunistic coinfections. sCD14 can contribute to cell activation by transferring monomeric LPS to membrane-bound CD14 or by directly transferring LPS to the MD-2/TLR4 complex. However, in the presence of high levels of LPS, sCD14 can also decrease LPS responsiveness [31]. Specifically, sCD14 can remove LPS from membrane-bound CD14 and divert it to plasma lipoproteins [32, 33]. It is also known that, in patients with HIV-1/AIDS, acute opportunistic coinfections are positively

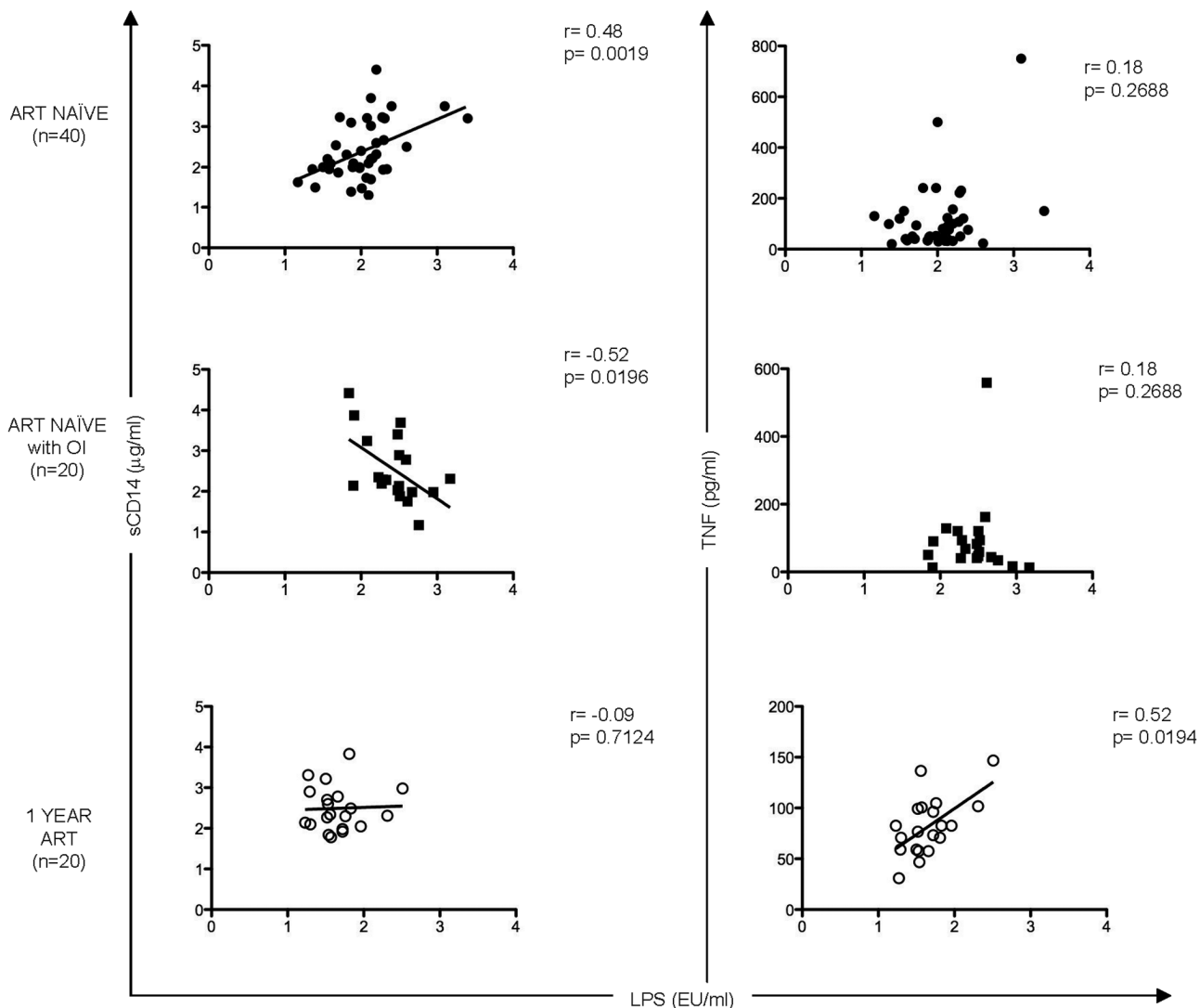


Figure 5. Associations between lipopolysaccharide (LPS) and plasma levels of soluble CD14 (sCD14) and tumor necrosis factor (TNF), before and during combination antiretroviral therapy (cART). Correlations were observed between LPS with sCD14 before cART and between LPS and TNF during cART. Levels of sCD14, TNF, and LPS were quantified in the plasma of chronically human immunodeficiency virus type 1 (HIV-1)-infected African patients with ($n = 40$) and without ($n = 20$) opportunistic coinfections (OIs) and in cART-treated (>1 year) patients ($n = 20$) with undetectable viral loads (ie, <50 HIV-1 RNA copies/mL for >6 months). Spearman correlations (r and P values) were calculated to determine the relationships between sCD14 and TNF levels and plasma LPS.

correlated with hyperlipidemia [34, 35]. These data suggest that, in the context of coinfections and high levels of LPS, sCD14 may shuttle LPS to lipoproteins, rapidly clearing this endotoxin from the circulation and limiting further inflammatory responses.

In addition to cytokines associated with microbial translocation, we found that some activation markers, including CCL2 and CXCL10, and the frequency of CD14⁺CD16⁺ monocytes, were directly linked to HIV-1 replication and were responsive to cART. The marked up-regulation of CCL2, CXCL10, and inflammatory (CD14⁺CD16⁺) monocytes before treatment and their parallel down-regulation in response to cART suggests that

they may be part of a common activation network involved in the recruitment and trafficking of proinflammatory cells and in the dissemination of virus to tissue sites. CD14⁺CD16⁺ monocytes produce high levels of inflammatory mediators [36, 37], including CCL2 and CXCL10, and are thought to be more susceptible to HIV-1 than CD16⁻ monocytes [38, 39]. CD14⁺CD16⁺ monocytes may also contribute to HIV-1-associated dementia by carrying virus into the central nervous system and differentiating into perivascular macrophages that secrete inflammatory cytokines and damage the underlying brain tissue [40–43]. Others have reported that CD14⁺CD16⁺ monocytes preferentially differentiate into macrophages that activate resting T cells for

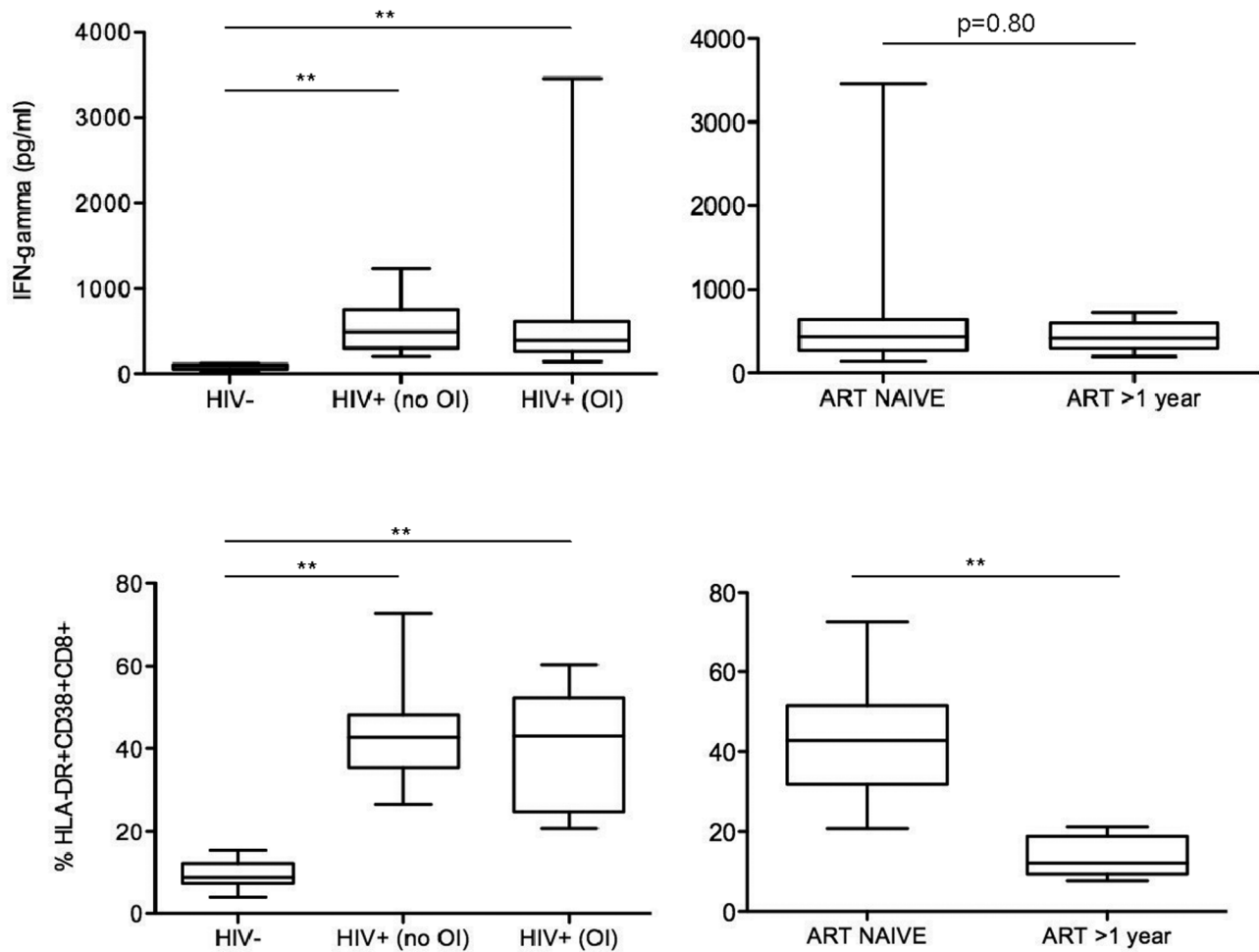


Figure 6. Markers of T cell activation and their response to combination antiretroviral therapy (cART). Interferon (IFN)- γ levels remained persistently elevated in patients receiving successful cART, despite a marked reduction in the frequency of activated HLA-DR⁺CD38⁺ CD8 T cells. Plasma levels of IFN- γ were quantified in cART-naive patients with ($n = 40$) and without ($n = 20$) opportunistic coinfections (OIs) and in uninfected African controls ($n = 10$). The frequency of HLA-DR⁺CD38⁺CD8⁺ T cells was determined by flow cytometry. Significant differences in IFN- γ levels and CD8 T cell activation, before and after cART, were determined using the Mann-Whitney test. ****** $P < .001$.

productive infection by secreting high levels of CCL2, CCL22, CCL24, and CCL17 [44, 45].

At the cytokine level, we found that the IL-6 level was increased in cART-naive patients with opportunistic coinfections but not in patients who lacked clinical evidence of an overt opportunistic coinfection, suggesting that the up-regulation of IL-6 was driven primarily by acute infections. This is in keeping with studies showing that IL-6, together with C-reactive protein, is an important mediator of acute phase responses [28, 29]. In this study, we examined the full spectrum of opportunistic coinfections present in our patient population rather than concentrating on specific coinfections, and as a result, our analyses were not powered to detect differences between individual opportunistic coinfections. Other studies, however, have reported that IL-6 levels are particularly high in HIV-1-

infected patients with pulmonary tuberculosis and *Mycobacterium avian* complex bacteremia [46, 47] and in patients with a history of immune restoration disease following the introduction of cART [48]. These data suggest that mycobacterium and undiagnosed subclinical infections are important determinants of IL-6 production in patients with chronic HIV-1 disease. *Mycobacterium* infections are common in South Africa and accounted for 3 (15%) of 20 coinfections in our cohort.

Despite a recent study linking heightened levels of plasma LPS and 16s ribosomal DNA to CD8 (and CD4) T cell hyperactivation [19], we did not observe any direct correlations between the frequency of CD38⁺HLADR⁺ CD8 T cells or IFN- γ and either plasma LPS, HIV-1 RNA, or monocyte activation, suggesting that multiple factors contribute to the increase in CD38, HLA-DR, and IFN- γ . The normalization of CD38 and

HLA-DR levels in patients receiving effective cART suggests that, in cART-naïve patients, HIV-1 plays a central role in the up-regulation of these activation markers. In contrast to CD38 and HLA-DR, IFN- γ remained persistently up-regulated in patients receiving cART. Although the source of this IFN- γ is not known, high levels of HIV-specific IL2⁺ and IFN- γ ⁺CD4⁺ T cells have been linked to controlled viral replication and immune reconstitution during cART [49].

These data underscore the importance of unraveling the complex interrelated correlations between immune activation, HIV-1 replication, subclinical endotoxemia, and acute opportunistic coinfections. We were able to dissect out clear-cut correlations between microbial translocation and immune activation in African patients with late-stage HIV-1 disease, both before and during cART, possibly because of the relative homogeneity of our patient cohort. Importantly, we found that subclinical endotoxemia was a dominant force driving chronic inflammation during cART. However, because of the cross-sectional nature of our study, we are unable to comment on the effects of microbial translocation on disease progression. In future studies, it will be important to determine whether persistent endotoxemia is linked to irreversible immune dysfunction and irreparable damage to the gastrointestinal tract or to residual low-level viral replication in gut-associated lymphoid tissue. Highly active antiretroviral therapy-treated patients with viral loads <2.5 copies/mL have been shown to have lower levels of microbial translocation, compared those with viral loads of 2.5–50 copies/mL [27]. Collectively, these data suggest that with enhanced therapy and intensified screening and treatment of opportunistic coinfections, it may be possible to improve treatment outcomes in African patients. Strategies aimed at preventing aberrant activation of the innate immune system may be particularly effective in this regard.

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