T Cell Activation in HIV-Seropositive Ugandans: Differential Associations with Viral Load, CD4+ T Cell Depletion, and Coinfection

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Immune activation is thought to play a major role in the pathogenesis of human immunodeficiency virus (HIV). This effect may be particularly relevant in Africa, where endemic coinfections may contribute to disease progression, perhaps as a consequence of enhanced immune activation. We investigated the expression of CD38 and human leukocyte antigen (HLA)–DR on T cells in 168 HIV-seropositive volunteers in Uganda. We observed higher levels of CD4+ and CD8+ T cell activation in Uganda, compared with those reported in previous studies from Western countries. Coexpression of CD38 and HLA-DR on both CD4+ and CD8+ T cell subsets was directly correlated with viral load and inversely correlated with CD4+ T cell counts. In antiretroviral therapy (ART)–naïve volunteers, viral load and CD4+ T cell count had stronger associations with CD8+ and CD4+ T cell activation, respectively. Virus suppression by ART was associated with a reduction in T cell activation, with a stronger observed effect on reducing CD8+ compared with CD4+ T cell activation. The presence of coinfection was associated with increased CD4+ T cell activation but, interestingly, not with increased CD8+ T cell activation. Our results suggest that distinct mechanisms differentially drive activation in CD4+ and CD8+ T cell subsets, which may impact the clinical prognostic values of T cell activation in HIV infection.

Immune activation has been suggested to be a stronger predictor of HIV disease progression than CD4+ T cell count or viral load alone [1–4] and may even predict antiretroviral therapy (ART) treatment failure [5, 6]. Despite the strong correlation between T cell activation and clinical outcome, the exact mechanism by which HIV activates the immune system remains unresolved. Direct antigen-driven T cell activation by HIV and coinfections, nonspecific bystander activation, and homeostatic proliferation have all been proposed as potential factors that drive immune activation [7–10]. Regardless of the mechanisms involved, T cell activation, and not direct viral infection of T cells, is believed to be the major cause of CD4+ T cell depletion in HIV infection, through a process of activation-induced cell death (AICD) [2, 7, 11–13].

Correlation of disease progression with immune activation previously has been established mainly in individuals infected with clade B viruses and primarily in developed countries. In sub-Saharan Africa, where non–clade B viruses predominate and where coinfection is frequent, relatively few studies have addressed the relevance of immune activation to HIV disease prognosis. Higher levels of T cell activation have been reported in HIV-seronegative Africans [14] and have been attributed to frequent infections by various pathogens endemic in this region. Indeed, several studies from Ethiopia have suggested that chronic immune activation associated with parasitic infections can drive CD4+ T cell depletion in HIV-seronegative individuals [3, 15, 16]. Given these observations, plus theoretical arguments that immune activation associated with endemic coinfections may promote more-rapid HIV disease progression in Africa [17, 18], we investigated the effect...
of HIV infection on immune activation in a cohort of HIV-seropositive Ugandans. The association between viral load, CD4+ T cell counts, and T cell activation was examined in the context of ART and HIV coinfection. We analyzed CD4+ and CD8+ T cell populations independently, to evaluate the hypothesis that factors mediating immune activation may differentially affect the activation state of these T cell subsets.

SUBJECTS, MATERIALS, AND METHODS

Study population and design. A total of 168 HIV-1–seropositive and 25 HIV-1–seronegative Ugandan adults visiting the HIV clinic at the Joint Clinical Research Centre in Kampala were enrolled in our cross-sectional study. Demographic information was compiled at the time of enrollment and blood draw. Volunteers were either ART naive or receiving treatment and were at all stages of disease. Diseases that are of high prevalence and endemic in Uganda were chosen as HIV–associated coinfections and were defined as clinically reported presence of *Mycobacterium tuberculosis*, cytomegalovirus (CMV), Cryptococcus neoformans, Candida albicans, Pneumocystis carinii, Toxoplasma gondii, herpes simplex virus, herpes zoster, malaria, or helminth infection (as reported by physicians at the time blood was drawn). All individuals with tuberculosis (TB) were in their second to ninth month of anti-TB therapy. Exclusion criteria included an age of <18 years, pregnancy, active TB (defined as suspected untreated TB or as TB during the first 2 months of anti-TB therapy), or moribund status. Institutional review board approval was obtained from the California Department of Health Services; the University of California, San Francisco; and the Joint Clinical Research Center, Kampala. All study participants gave written, informed consent.

T cell immunophenotyping. Freshly isolated peripheral blood mononuclear cells (PBMCs) were analyzed for CD4+ and CD8+ T cell activation by use of the Beckton Dickinson FACSCalibur. Cells were stained with fluochrome monoclonal antibodies: CD4 or CD8 PerCP-cy5.5, HLA-DR phycoerythrin (PE), and CD38 allophycocyanin (APC) (BD Pharmingen). Analysis was performed with FLOWJO software (version 4.5.9; Treestar). Preset gating was applied to all samples and was based on the expression of activation markers in HIV-seronegative Ugandans (see figure A1 in the Appendix, which appears only in the electronic edition of the *Journal*). Validation of the described staining protocols, FACSCalibur settings, and gating analyses was performed using a separate FACSCalibur in the United States. US HIV-seronegative samples and cryopreserved Ugandan PBMC samples were analyzed concomitantly, to ensure consistency of settings between sites. These independent analyses demonstrated that the immune activation levels for US HIV-seronegative samples are similar to those reported in previous studies of HIV-seronegative individuals from Western countries (0.5% and 1.1%, for CD4+ and CD8+ T cells, respectively). Comparable activation levels from the Ugandan samples were obtained from assays performed in the United States and Uganda. Absolute numbers of CD4+ or CD8+ T cells were determined by use of Becton Dickinson TruCount. More than 1000 CD4+ and CD8+ T cell events were acquired for all analyses.

Plasma viral load. Viral load (HIV-1 RNA levels) in plasma was determined by use of the Roche Amplicor 1.5. Since the sensitivity of the assay is reported to be 400 copies/mL, all undetectable levels were assigned a value of 0. In addition, all values >750,000 copies/mL were assigned a value of 750,000 copies/mL, per the manufacturer’s instructions.

Statistical analysis. A linear least-squares regression model was used for statistical analysis. Variables whose values were nonlinearly distributed were log transformed, to permit the use of the linear model. All models were verified by viewing residual plots to ensure that the basic assumptions of linear regression were not violated and to ensure that log transformation was appropriate. Statistical significance was defined as *P* < .05. To highlight the contribution of each independent variable to the dependent variables, we calculated the increment change (expressed as a percentage) from individual estimate values (univariate and multivariate) of the dependent variable when CD4+ T cell count was increased by 100 cells/mm3, when viral load was doubled, or when a coinfection was present. Relative strengths of associations between factors were calculated by measuring the incremental contribution of the individual variables to the *R*2 of the final model.

RESULTS

Baseline demographic and clinical characteristics of the volunteers. A total of 168 HIV-seropositive volunteers were enrolled from an HIV referral center in Kampala. The average age of volunteers was 38 years (range, 21–73 years), and 60% of volunteers were female. The median plasma viral load of volunteers was 105,996 copies/mL (interquartile range, 10,179–320,000 copies/mL), and the median CD4+ T cell count was 186 cells/mm3 (interquartile range, 88–297 cells/mm3). Seventy-five percent of volunteers were ART naive. Of the participants receiving ART, 42% had undetectable viral loads. On the basis of World Health Organization clinical classification the participants’ HIV disease stages were as follows: 8% had stage 1 disease, 32% stage 2, 40% stage 3, 10% stage 4, and 10% stage “unknown.” Twenty-eight percent of the cohort had physician-reported presence of at least 1 coinfection at the time that blood was drawn. TB was the most common coinfection (40%), followed by candida (30%), malaria (13%), and herpes zoster (13%). All treated individuals were receiving a regimen containing nucleoside analogues; 82% were also receiving a non-nucleoside reverse-transcriptase inhibitor, and 16% were receiving a protease inhibitor.

Twenty-five healthy HIV-seronegative adult Ugandans were
enrolled as control subjects. The average age of control subjects was 32 years (range, 19–50 years), and 37% were female. The median CD4+ T cell count, in those who had CD4+ T cell counts evaluated (n = 11), was 704 cells/mm3 (range, 547–1618 cells/mm3). This range of CD4+ T cell counts is similar to that reported in previous studies from Uganda [19].

**Immune activation in HIV-seropositive and -seronegative Ugandans.** Activated T cells were defined by coexpression of CD38 and HLA-DR (see figure A1 in the Appendix, which appears only in the electronic edition of the Journal). To avoid intersample variability, all gates were preset and define using HIV-seronegative samples. HIV-seronegative Ugandans (n = 25) had median levels of CD4+ and CD8+ T cell activation (5% and 13%, respectively) similar to those reported in previous studies of HIV-seronegative Ethiopians (7%–8% and 12%–15%, respectively) [14] (table 1). The median levels of CD4+ and CD8+ T cell activation were high in ART-naive HIV-seropositive individuals (28% and 65%, respectively) and were significantly different from those in HIV-seronegative Ugandans (P < .0001, for both CD4+ and CD8+ T cell activation) (table 1).

**Effect of ART on CD4+ and CD8+ T cell activation.** We next investigated the effect of ART on T cell activation. Compared with ART-naive individuals, study participants receiving ART who had undetectable viral loads demonstrated substantially lower levels of activation for both CD4+ and CD8+ T cells (15% and 30%, respectively) (table 1). This difference was highly significant for each comparison (P < .0001, for both CD4+ and CD8+ T cell activation in untreated patients vs. patients with viral suppression). In contrast, patients receiving ART who did not achieve complete viral suppression did not demonstrate lower activation of CD4+ or CD8+ T cells, compared with ART-naive individuals (P = .98 and P = .60, respectively). In addition, study participants receiving ART who had undetectable viral loads had significantly higher levels of both CD4+ and CD8+ T cell activation, compared with HIV-seronegative Ugandan volunteers (P < .0001, for both CD4+ and CD8+ T cells).

**Factors associated with T cell activation.** We next evaluated the associations between CD4+ T cell count, viral load, and T cell activation in ART-naive individuals. We found a significant inverse correlation between CD4+ T cell count and CD4+ and CD8+ T cell activation (figure e1A and 1B) and a direct correlation between viral load and CD4+ and CD8+ T cell activation (figure e1C and 1D) in univariate analysis. These relationships remained statistically significant whether we analyzed ART-naive individuals alone or all study participants (data not shown). We next investigated whether coinfection is associated with CD4+ or CD8+ T cell activation. We observed a significant direct correlation between the presence of a coinfection and CD4+, but not CD8+, T cell activation (P = .02 and P = .24, respectively; table 2) in univariate analysis. No significant association between CD4+ or CD8+ T cell activation and CD4+ T cell count was observed in healthy HIV-seronegative volunteers (P = .34 and P = .86, respectively).

Multivariate analyses were performed to further investigate whether viral load, CD4+ T cell depletion, or presence of coinfection exert independent effects on T cell activation (table 2). Viral load was associated with both CD4+ and CD8+ T cell activation in both univariate and multivariate analyses controlling for CD4+ T cell count and coinfection. In contrast, although CD4+ T cell count was associated with CD4+ and CD8+ T cell activation in univariate analysis, correlation with CD8+ T cell activation was lost when the analysis was controlled for viral load and coinfection. The association between coinfection and CD4+ T cell activation remained statistically significant in multivariate analysis. No statistically significant association was apparent between CD8+ T cell activation and coinfection. Although we observed no significant associations between individual coinfections and T cell activation (data not shown), TB appeared to be closely associated with CD4+ T cell activation in multivariate models; however, this effect did not reach statistical significance (R² = .47; P = .06).

**Independent expression of individual activation markers on T cell subsets.** We next investigated individual expression of CD38 and HLA-DR on T cell subsets. For ease of comparison, coefficient were transformed to reflect the change in activation when CD4+ T cell count increased by 100 cells/mm³, when viral load was doubled, or when coinfection was present. We found that CD38 expression on both CD4+ and CD8+ T cells was significantly associated with viral load in univariate and multivariate analysis (table 2). In addition, HLA-DR expression on CD4+ T cells was correlated with CD4+ T cell count and viral load. In contrast, HLA-DR expression on CD8+ T cells was not correlated with viral load or CD4+ T cell count in univariate

<table>
<thead>
<tr>
<th>Table 1. CD4+ and CD8+ T cell activation by HIV status.</th>
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<td><strong>Group</strong></td>
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<tr>
<td>HIV seronegative</td>
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<tr>
<td>HIV seropositive</td>
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<tr>
<td>Receiving ART</td>
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<td>VL &gt;400 copies/mL</td>
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</tbody>
</table>

**NOTE.** ART, antiretroviral therapy; IQR, interquartile range; VL, viral load.

* P < .0001, for both CD4+ and CD8+ T cell activation vs. the HIV-seropositive ART-naive group and vs. the HIV-seropositive group receiving ART with undetectable VL.

b P = .0002 and P < .0001, for CD4+ and CD8+ T cell activation, respectively, vs. the HIV-seropositive ART-naive group.

c P = .98 and P = .60, for CD4+ and CD8+ T cell activation, respectively, vs. the HIV-seropositive ART-naive group.
or multivariate analyses. Interestingly, in both univariate and multivariate models, coinfection was associated with increased HLA-DR expression only on CD4⁺, but not CD8⁺, T cells.

**Relative effect of CD4⁺ T cell count and viral load on T cell activation.** The relative strengths of the associations of viral load and CD4⁺ T cell count with T cell activation was examined by measuring the contribution of these factors to the increment increase in the $R^2$ value, by use of the regression analysis model. We first focused on the ART-naive cohort and calculated incremental changes in $R^2$ values for CD4⁺ T cell count and viral load when subsequent variables were added to the model. As shown in table 3, CD4⁺ T cell count was more strongly associated with CD4⁺ T cell activation than with CD8⁺ T cell activation when viral load was controlled for ($R^2$ increment of .19 vs .02, respectively). In contrast, viral load had a stronger association with CD8⁺ T cell activation when CD4⁺ T cell count was controlled for ($R^2$ increment of .12 vs .03). We next measured the association of ART with T cell activation. Controlling for CD4⁺ T cell count, we found that, when viral load was fully suppressed, the incremental increases in $R^2$ values were .12 and .30 for CD4⁺ and CD8⁺ T cell activation, respectively, suggesting that ART has a stronger effect on CD8⁺ T cell activation (table 3).

**DISCUSSION**

Immune activation is predictive of HIV disease progression in ART-naive populations and has been associated with lower
gains in CD4+ T cell counts after initiation of ART [4, 6, 20–22]. However, these significant associations mostly have been studied in North America and Europe, where HIV-1 clade B viruses predominate [1, 4–6, 20, 23–26]. The factors that influence immune activation and its role as a clinical predictor in sub-Saharan Africa, where non-clade B viruses predominate and coinfection is prevalent, has yet to be established. In a cross-sectional study of HIV-seropositive Ugandan adults, we found dramatically elevated levels of T cell activation and investigated factors that may be responsible. We demonstrated that viral load, CD4+ T cell count, and coinfection are all strongly associated with T cell activation in this population. Interestingly, each factor was differentially associated with individual activation markers and also had distinct associations with CD4+ and CD8+ T cell lineages.

The level of immune activation in HIV-seronegative volunteers in our Ugandan study population is up to 3-fold higher than those previously observed in HIV-seronegative cohorts from the United States or Europe [27, 28]. In this regard, our finding are in agreement with those of previous studies demonstrating higher levels of T cell activation in HIV-seronegative Africans [17, 29–31]. On the basis of our analysis, we cannot conclude whether this observed phenomenon is a consequence of environmental factors, including endemic infection, or whether genetic influence are partly responsible.

The immune activation profile in our cohort of HIV-seropositive Ugandans shows a striking increase in CD4+ and CD8+ T cell activation, much higher than that previously reported in the United States or Europe [2, 20, 28, 32, 33], although direct comparisons between studies are complicated by differences in analyses. HIV-seropositive Ugandan volunteers have nearly 2-fold higher levels of T cell activation than do HIV-seropositive volunteers in more-developed regions. Lower CD4+ T cell counts, higher viral loads, and the presence of frequent endemic coinfection likely contribute to the observed high levels of activation.

Our study demonstrated that both CD4+ T cell count and viral load had significant associations with both CD4+ and CD8+ T cell activation, similar to what was found in previous studies [7, 20]. However, our data revealed that viral load and CD4+ T cell count show different degrees of association with CD4+ and CD8+ T cell subsets. Whereas viral load had a much stronger association with immune activation of CD8+ T cells, CD4+ T cell count had a greater correlation with activation of CD4+ T cells. In fact, the correlation between CD8+ T cell activation

Table 2. Associations between clinical factors and immune activation.

<table>
<thead>
<tr>
<th>Variablea</th>
<th>Dependent</th>
<th>Independent</th>
<th>Change in estimate (95% CL), %</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Univariate</td>
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<tr>
<td>CD4+ T cell count</td>
<td>HLA-DR and CD38 expression on CD4+ T cells</td>
<td>Viral load</td>
<td>−18.1b (−22.3, −13.7)</td>
</tr>
<tr>
<td>CD4+ T cell count</td>
<td></td>
<td>Coinfection</td>
<td>10.2b (7.1, 13.3)</td>
</tr>
<tr>
<td>CD4+ T cell count</td>
<td>HLA-DR and CD38 expression on CD8+ T cells</td>
<td>Viral load</td>
<td>−5.8c (−8.9, −2.7)</td>
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<tr>
<td>CD4+ T cell count</td>
<td></td>
<td>Coinfection</td>
<td>7.9b (6.3, 9.5)</td>
</tr>
<tr>
<td>CD8+ T cell count</td>
<td>CD38 expression on CD4+ T cells</td>
<td>Viral load</td>
<td>−0 (−2.3, 2.3)</td>
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<tr>
<td>CD8+ T cell count</td>
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<td>Coinfection</td>
<td>−2.0 (−11.6, 8.7)</td>
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<tr>
<td>CD8+ T cell count</td>
<td>CD38 expression on CD8+ T cells</td>
<td>Viral load</td>
<td>−4.9b (−6.9, −2.8)</td>
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<tr>
<td>CD8+ T cell count</td>
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<td>Coinfection</td>
<td>5.0b (3.9, 6.1)</td>
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<tr>
<td>CD8+ T cell count</td>
<td>HLA-DR expression on CD4+ T cells</td>
<td>Viral load</td>
<td>−18.1b (−21.3, −14.8)</td>
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<tr>
<td>CD8+ T cell count</td>
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<td>Coinfection</td>
<td>5.0b (2.5, 7.5)</td>
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<tr>
<td>CD8+ T cell count</td>
<td>HLA-DR expression on CD8+ T cells</td>
<td>Viral load</td>
<td>−10.5 (−19.6, 1.8)</td>
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<tr>
<td>CD8+ T cell count</td>
<td></td>
<td>Coinfection</td>
<td>6.4 (−0.4, 13.8)</td>
</tr>
</tbody>
</table>

**NOTE.** CL, confidence limits; HLA-DR, human leukocyte antigen–DR. Data are the calculated percentage change when CD4+ T cell count was increased by 100 cells/mm³, when viral load was doubled, or when a coinfection was present.

*a* All values of dependent variables and viral load were log transformed.

*P < .0001*

*P < .05*
Table 3. Relative effect of viral load and CD4+ T cell depletion on immune activation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dependent</th>
<th>Independent</th>
<th>R²</th>
<th>Change in R²</th>
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<td>CD4+ T cell count (viral load suppressed by ART)</td>
<td>0.07</td>
<td>-0.30</td>
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</table>

NOTE. ART, antiretroviral therapy.

a ART-naive subjects only.
b Controlled for CD4+ T cell count.
c Reference value.

and CD4+ T cell count was no longer significant in our cohort in multivariate analyses after viral load was controlled for. This suggests that previously reported associations between CD4+ T cell depletion and CD8+ T cell activation, which were based on coexpression of HLA-DR and CD38, may be indirect and attributable to the relationship between increasing viral loads and decreasing CD4+ T cell counts. Consistent with the finding that viral load preferentially modulates CD8+ T cell activation, viral suppression by ART was found to have a stronger association with reduced CD8+ T cell activation in our study. These observed differences are potentially attributable to the distinct proliferative capacity and homeostatic proliferation pathways for CD4+ and CD8+ T cells [34, 35]. However, it is likely that complex factors mediate these differential effects on T cell subset activation.

In addition to viral load and CD4+ T cell count, coinfection is associated with changes in CD4+ T cell activation profiles and this effect appears to be independent of these other factors. In contrast, coinfection had no statistically significant association with CD8+ T cell activation in multivariate analysis, although there was a trend in the same direction. Activation of the immune system by exogenous stimuli, such as immunizations and copathogens, may enhance HIV replication, increase plasma viral load [36–38], and worsen prognosis [39]. Since we observed an association between coinfection and CD4+ T cell activation that was independent of viral load and CD4+ T cell count, our data suggest that the presence of coinfection enhances immune activation directly, and not indirectly through increased levels of HIV replication or stages of immunodeficiency.

Numerous studies have confirmed that TB and sexually transmitted diseases (STDs) are associated with increased viral load; however, data concerning the effect of coinfection on immune activation have been conflicting [18, 40–42]. For example, whereas coinfection with STDs [43] and TB [41, 44] have not been associated with increased immune activation in several recent studies in Africa, hepatitis C has been linked to increased CD4+ and CD8+ T cell activation in the United States [6]. Our data suggest that the association between CD4+ T cell activation and coinfection is primarily driven by HLA-DR expression. These findings are consistent with the observation by Orendi et al. that patients receiving highly active ART who have an opportunistic infection have increased HLA-DR expression on CD4+ T cells but no change in HLA-DR or CD38 expression on CD8+ T cells [20].

In our study, the association between HIV coinfection and CD4+ T cell activation alone suggests that infections endemic in Uganda might play a role in AICD-mediated CD4+ T cell depletion. In addition, it raises the concern that coinfection could represent a major confounding variable in CD4+ specific activation analysis. Whether coinfections are acting additively or synergistically with HIV to increase immune activation remains unresolved. Interestingly, we did not observe an association between immune activation and CD4+ T cell count in healthy HIV-seronegative Ugandans. It is possible that the effect on activation requires preexisting HIV infection. Alternatively, the lower CD4+ T cell counts in Ugandans might be due to a cumulative history of numerous infections that would not necessarily be reflected in the activation state at the time that blood was drawn. The mechanism surrounding preferential activation of CD4+, but not CD8+, T cells by coinfections is also unclear and may depend on the type of pathogen present. Perhaps viral coinfections, such as with hepatitis C virus, have stronger effects on CD8+ T cells, whereas other pathogens have more of
an effect on CD4+ T cells. In the Ugandan population in our study, the predominant HIV coinfections were parasitic, fungal, and mycobacterial, which may explain the stronger associations with CD4+ T cell activation. The sample size and the physician-based reporting of coinfection in our study did not allow further examination of whether specific endemic diseases or repeated coinfections contributed to the observed elevated immune activation.

Several immunophenotypic markers have been used to evaluate T cell activation ex vivo, although HLA-DR and CD38 are probably the most well-characterized markers of immune activation in HIV infection. Interestingly, individual analysis of CD38 and HLA-DR in the population in our study suggests that not only do these 2 activation markers have distinct associations with viral load, CD4+ T cell count, and coinfection, but they also behave differently on CD4+ and CD8+ T cells. CD38 appears to be up-regulated on both CD4+ and CD8+ T cells in response to viral load, but its expression is not associated with CD4+ T cell count in either subset in multivariate analysis. In contrast, HLA-DR up-regulation on CD4+ T cells is associated with viral load, CD4+ T cell count, and presence of coinfection, with no correlations in the CD8+ T cell population. Thus, the use of HLA-DR up-regulation as a single activation immunophenotype for CD8+ T cells may be less valid as a prognostic marker in HIV infection. Reliance on immune activation profile to provide cost-effective information on clinical prognosis for HIV infection in Africa clearly will require further examination of the multiple factors influencing the differential expression of these markers on T cell subsets.

Our study reports some of the highest levels of immune activation in HIV infection and addresses the relatively understudied area of immune activation in sub-Saharan Africa [29, 45]. HIV infection in Uganda is caused by multiple endemic viral strains (subtypes A, C, and D [46, 47]), and it is unclear whether this contributes to the high level of activation observed in our study. Certainly, the increased prevalence of coinfection and higher baseline activation levels also play a role. In addition, it is unclear whether the increased activation we observe will influence HIV disease outcome and, perhaps, response to treatment in Africa. A longitudinal study in these regions of sub-Saharan Africa that addresses these factors will be necessary before the true impact of immune activation and its contribution to disease progression can be assessed.

Acknowledgments

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