# The effect of HIV infection and HAART on inflammatory biomarkers in a population-based cohort of women

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**Objective:** HIV causes inflammation that can be at least partially corrected by HAART. To determine the qualitative and quantitative nature of cytokine perturbation, we compared cytokine patterns in three HIV clinical groups, including HAART responders (HAART), untreated HIV noncontrollers, and HIV-uninfected (NEG).

**Methods:** Multiplex assays were used to measure 32 cytokines in a cross-sectional study of participants in the Women's Interagency HIV Study. Participants from three groups were included: HAART ($n = 17$), noncontrollers ($n = 14$), and HIV NEG ($n = 17$).

**Results:** Several cytokines and chemokines showed significant differences between noncontrollers and NEG participants, including elevated interferon gamma-induced 10 (IP-10) and tumor necrosis factor-α (TNF-α) and decreased interleukin-12(p40) [IL-12(p40)], IL-15, and fibroblast growth factor-2 (FGF-2) in noncontroller participants. Biomarker levels among HAART women more closely resembled the NEG, with the exception of TNF-α and FGF-2. Secondary analyses of the combined HAART and noncontroller groups revealed that IP-10 showed a strong, positive correlation with viral load and negative correlation with $CD4^+$ T-cell counts. The growth factors vascular endothelial growth factor, epidermal growth factor, and FGF-2 all showed a positive correlation with increased $CD4^+$ T-cell counts.

**Conclusion:** Untreated, progressive HIV infection was associated with decreased serum levels of cytokines important in T-cell homeostasis (IL-15) and T-cell phenotype determination (IL-12), and increased levels of innate inflammatory mediators such as IP-10 and TNF-α. HAART was associated with cytokine profiles that more closely resembled those of HIV-uninfected women. The distinctive pattern of cytokine levels in the three study groups may provide insights into HIV pathogenesis, and responses to therapy.

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## Introduction

Infection with HIV leads to immune dysfunction and progression to AIDS within 10–11 years in the absence of antiretroviral therapy in the majority of infected persons [1,2]. There is considerable variability in the rate of disease progression, and the factors underlying the pathogenesis of HIV are not entirely understood. Persons with progressive HIV infection on average have higher viral loads [3] and elevated levels of activated T cells [3,4], including HIV-specific T cells [5]. Elevated cytokine levels in HIV infection could have positive or negative effects on viral control or $CD4^+$ T-cell homeostasis. In-vitro studies have revealed that a number of factors can contribute to enhanced HIV replication, including tumor necrosis factor-α (TNF-α) [6–8] and interleukin-2 (IL-2) [9,10]. In contrast, some cytokines appear to decrease HIV replication in tissue culture, including interferon-α (IFN-α) [11,12], IFN-γ [6,12,13], and granulocyte-macrophage colony-stimulating factor (GM-CSF) [13].

Prior studies have assessed the associations of soluble markers of inflammation with disease outcome during chronic simian immunodeficiency virus (SIV) or HIV infection. An SIV model of infection revealed no differences in cytokine perturbations between animals that progressed rapidly vs. slowly to AIDS when studied at the terminal stage of AIDS, though animals with encephalitis had higher IL-2 and IL-6 levels compared to animals without encephalopathy [14]. Recent studies of pathogenic vs. nonpathogenic infection of rhesus macaques and African green monkeys have revealed a host of factors differentially regulated in these disease models [15–18]. In humans, prior work has demonstrated that patients with chronic HIV infection typically show elevations in serum or plasma TNF-α levels [19–21]. In a large cohort of HIV-infected men, the plasma activation markers soluble TNF receptor II (sTNF-RII), neopterin, and sIL-2R correlated well with each other and had some ability to predict progression to AIDS independent of $CD4^+$ T-cell count or plasma HIV viral load [22]. More recent data from the Strategies for Management of Anti-Retroviral Therapy (SMART) trial showed that elevated levels of C-reactive protein, IL-6, and D-dimer were associated with increased risk of death in a cohort in which most participants received HAART [23].

The above studies suggest that soluble mediators of inflammation are associated with HIV disease progression. Our group and others have identified soluble markers of inflammation that are dysregulated in acute HIV infection [24,25], more recently using newly available multiplex cytokine testing reagents [26,27]. We applied these multiplex cytokine detection techniques to study HIV-seronegative women and women with chronic HIV infection. Thirty-two soluble immune markers were quantified and correlated with clinical group, viral load, and $CD4^+$ T-cell count. This study furnishes a more complete picture of the degree and importantly the breadth of immune dysfunction associated with uncontrolled viral replication and reveals the ability and limitations of HAART to correct the systemic inflammation associated with HIV.

## Methods

### Study participants

Study participants were individuals in the Women's Interagency HIV Study (WIHS), an ongoing multisite cohort study of HIV among US women, which enrolled participants in 1994–1995 and 2001–2002 [28,29]. Semiannual visits include interview, clinical examination, and collection of biologic specimens. HIV noncontrollers ($n = 14$) were antiretroviral therapy naive and had a viral load more than 10 000 RNA copies/ml for at least one of two time points separated by 6 months. HAART responders ($n = 17$) had undetectable viral load (<80 RNA copies/ml) for at least 12 months while on a potent combination antiretroviral regimen. HIV-uninfected women (NEG, $n = 17$) in WIHS undergo the same follow-up procedures as the HIV-infected women and have HIV serology performed every 6 months. Hepatitis C virus (HCV) serology was performed at study entry, and HCV plasma RNA quantitation was performed on seropositive women to determine whether infection was ongoing vs. resolved. Participants for the current study were chosen from the total WIHS cohort of 3766 women to match within the three study groups (NEG, HAART, and noncontroller) based on ethnicity (African–American vs. other), age, BMI, HCV antibody status at study entry, and time of follow-up in the cohort (within 1 year).

### Sample selection

Two serum samples for each participant were tested, with the samples chosen near the beginning and end of the period of clinical interest (i.e., during the period of undetectable viremia for the HAART group and during a period of the highest level viremia for the noncontroller group). The average time span between the paired samples from each participant was 2.7, 3.3, and 2.9 years for the NEG, HAART, and noncontroller groups, respectively. For three HAART participants, one of the two samples tested was plasma, and these samples were excluded from the analysis due to potential differences in cytokine levels between these two sample types (data not shown).

### Multiplex cytokine and chemokine analysis

Serum samples were assayed using the high-sensitivity LincoPlex kit (Millipore, Billerica, Massachusetts, USA) for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 p70, IL-13, IFN-γ, GM-CSF, and TNF-α, and the standard-sensitivity Milliplex Map kit (Millipore) for

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epidermal growth factor (EGF), Eotaxin, fibroblast growth factor (FGF)-2, fractalkine, IL-1α, IL-1Rα, IL-9, IL-12(p40), IL-15, IL-17, IP-10, monocyte chemotactic protein (MCP)-1, MCP-3, macrophage-derived chemokine (MDC), macrophage inflammatory protein (MIP)-1α, MIP-1β, sIL-2Rα, TNF-β, and vascular endothelial growth factor (VEGF) following the manufacturer's protocols. Standard curves were run in duplicate, and samples were tested in duplicate. Samples were acquired on a Labscan 100 analyzer (Luminex Corp., Austin, Texas, USA) using Bio-Plex manager 4.1 software (Bio-Rad Life Science, Hercules, California, USA). FGF-2 levels were also assayed in secondary testing by high-sensitivity ELISA (R&D Systems, Minneapolis, Minnesota, USA) according to manufacturer's instructions.

### Statistical analysis

For participants with data available from two time points (all but the three HAART participants noted above), cytokine levels were averaged over the two time points for analysis of the association of cytokine levels with clinical group. For analyses of cytokine level correlations with viral load or $CD4^+$ T-cell count, the observations from each participant were not averaged. Cytokine levels were compared between clinical groups using one-way analysis of variance (ANOVA) and Tukey's HSD (Honestly Significant Differences) tests. Differences in participant characteristics between groups were evaluated by ANOVA for continuous variables and by Fisher's exact test for ongoing HCV infection. Associations of cytokine levels with HIV viral load and $CD4^+$ T-cell count were assessed via linear regression. For analyses that included viral load values, these were set at 40 RNA copies/ml for participants with an undetectable viral load (half the lower limit of detection of 80 RNA copies/ml). Cytokine and viral load values were log-transformed prior to analysis due to nonnormal distribution of the data. $P$ values were adjusted into FDRs (false discovery rates) by the Benjamini and Hochberg controlling procedure [30], a commonly used method for analysis of large sets of biological data. Statistical significance was defined as $P$ less than 0.05 and FDR less than 0.1. R/Bioconductor software was utilized for analyses.

## Results

### Participant characteristics

Consistent with the overall WIHS cohort, each of the study groups (NEG, HAART, and noncontroller) was predominately African–American (Table 1). The median age for each group at the time of sample collection was 35–40 years and did not differ significantly between groups ($P = 0.3$, ANOVA). Median $CD4^+$ T-cell counts were significantly lower in the noncontroller group compared to NEG and HAART women ($P < 0.001$, ANOVA). The difference in $CD4^+$ T-cell counts between the NEG and HAART was not significant ($P > 0.05$, Tukey's posttest).

### Cytokine perturbations are most pronounced in viremic women

Multianalyte bead-based assays were used to assess 32 soluble markers covering pro-inflammatory and anti-inflammatory mediators, chemotaxis signaling, and growth factor secretion. The vast majority of analytes tested (27 of 32) did not show significant differences between the study groups (Table 2). However, five analytes were significantly different in one or both groups of HIV-infected women. Compared to HIV-negative women, noncontroller participants had significantly higher serum levels of the inflammatory mediators TNF-α and IP-10 and significantly lower serum levels of IL-12(p40), IL-15, and FGF-2 (Fig. 1). Levels of the analytes also differed at the level of statistical significance after application of a FDR step-down procedure to the dataset to account for the multiple analytes examined [30].

One possible confounder of the results would be ongoing HCV replication, which could drive soluble markers of inflammation. We first examined cytokine levels in HCV RNA-positive vs. HCV RNA-negative women within the HIV NEG and HAART groups. Levels of IP-10 were significantly higher in both groups of HCV viremic women, and IL-10 was higher only in the HIV NEG, HCV RNA-positive women ($P < 0.05$, FDR $< 0.1$). To control for this, the results were re-analyzed, excluding

**Table 1. Demographic and clinical characteristics in 48 included women.**

| | HIV-negative | HAART | Noncontrollers | *P* value |
|---|---|---|---|---|
| Age (years) | 35 (33–45) | 40 (36–46) | 38 (34–44) | 0.3 |
| Race (% Black) | 82% | 82% | 79% | 1 |
| BMI | 28.8 (24.2–36.3) | 29 (26.7–32.7) | 31.9 (24.8–39.4) | 0.2 |
| IDU | 3 (18%) | 3 (18%) | 1 (7%) | 0.6 |
| HCV antibody+ | 4 (24%) | 6 (35%) | 2 (14%) | 0.4 |
| HCV RNA+ | 3 (18%) | 3 (18%) | 0 (0%) | 0.2 |
| CD4 cell count (cells/μl) | 908 (703–1193) | 841 (703–1042) | 553 (381–756) | <0.0001 |
| Viral load (RNA copies/ml) | | <80 | 14 000 (6200–35 000) | N/A |

Median and interquartile range (IQR) values are shown, except race which is shown as percentage. HCV RNA+, hepatitis C virus RNA positive at study entry; IDU, self-reported intravenous drug use at study entry.

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Table 2. Cytokine levels by disease category.

| | NEG | | HAART | | Noncontrollers | |
|---|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | Median | IQR |
| Pro-inflammatory/T cell | | | | | | |
| IL-1α | 38.6 | 1.6–134 | 1.6 | 1.6–75 | 1.6 | 1.6–1.6 |
| IL-1β | 0.1 | 0.1–0.7 | 0.1 | 0.1–0.1 | 0.1 | 0.1–0.1 |
| IL-2 | 2.8 | 0.3–8.6 | 0.3 | 0.1–0.5 | 0.3 | 0.1–1.3 |
| IL-6 | 3.3 | 2.1–5.8 | 2.2 | 1–5.1 | 2.2 | 1.2–4.8 |
| IL-7 | 7.2 | 5.0–9.3 | 8.8 | 5.7–9.7 | 7.6 | 5.4–13.9 |
| IL-8 | 22.8 | 6.3–50.1 | 13.7 | 6.4–22.4 | 11.4 | 7.1–20.7 |
| IL-9 | 6.8 | 1.6–80.6 | 1.6 | 1.6–5.9 | 2.1 | 1.6–19.4 |
| **IL-12(p40)** | 67.2 | 3.6–244 | 1.6 | 1.6–93.9 | **1.6** | 1.6–1.6 |
| IL-12(p70) | 0.2 | 0.1–2.7 | 0.5 | 0.1–1.2 | 0.1 | 0.1–0.2 |
| **IL-15** | 5.8 | 3.8–32.1 | 5.3 | 2.7–5.9 | **1.6** | 1.6–2.7 |
| IL-17 | 6.4 | 2.0–58.7 | 8.2 | 1.6–45.1 | 1.6 | 1.6–2.5 |
| IFN-γ | 1.8 | 0.1–3.4 | 0.2 | 0.1–0.5 | 0.1 | 0.1–1.1 |
| **TNF-α** | 4.7 | 3.9–7.3 | **10.4** | 9.2–14.1 | **12.7** | 11.4–18.2 |
| TNF-β | 1.6 | 1.6–11.1 | 1.6 | 1.6–1.6 | 1.6 | 1.6–1.6 |
| GM-CSF | 1.6 | 0.6–3.3 | 0.2 | 0.1–1.3 | 0.2 | 0.1–1.3 |
| Anti-inflammatory/Th2 | | | | | | |
| IL-1Rα | 8.2 | 1.6–75.3 | 1.6 | 1.6–10.4 | 1.6 | 1.6–24.5 |
| sIL-2Rα | 23.5 | 1.6–53.1 | 77.8 | 55.8–189 | 46.8 | 4.3–120 |
| IL-4 | 0.2 | 0.1–5.8 | 0.3 | 0.1–0.3 | 0.3 | 0.1–8.9 |
| IL-5 | 0.1 | 0.1–0.5 | 0.1 | 0.1–0.3 | 0.2 | 0.1–0.5 |
| IL-10 | 6.4 | 3.4–12 | 7.5 | 3.3–9.7 | 12.1 | 6–16 |
| IL-13 | 2.7 | 1.3–10.2 | 0.3 | 0.2–5.3 | 1.1 | 0.1–8.1 |
| Chemoattractants | | | | | | |
| **IP-10** | 143 | 110–224 | 204 | 146–322 | **514** | 412–773 |
| MCP-1 | 411 | 307–560 | 552 | 393–675 | 541 | 445–633 |
| MCP-3 | 29.4 | 2.5–58.5 | 1.6 | 1.6–19 | 1.6 | 1.6–32.6 |
| MDC | 2570 | 1990–3460 | 3460 | 2910–4140 | 2720 | 2020–3060 |
| MIP-1α | 125 | 69.1–242 | 71 | 29.2–128 | 68.3 | 35.8–144 |
| MIP-1β | 74.0 | 52.3–195 | 82 | 60.3–140 | 56.1 | 48.9–78.1 |
| Eotaxin | 93.2 | 60.3–160 | 80.3 | 73.6–165 | 95.8 | 71.6–138 |
| Fractalkine | 35.6 | 1.6–278 | 64.7 | 1.6–400 | 1.6 | 1.6–50.1 |
| Growth factors | | | | | | |
| VEGF | 222 | 122–404 | 360 | 116–544 | 194 | 156–272 |
| EGF | 167 | 120–245 | 199 | 134–291 | 208 | 121–247 |
| **FGF-2** | 32.8 | 18.6–73.2 | **13.8** | 1.6–23.4 | **8.3** | 1.6–17.8 |

Bold numbers denote values different from HIV NEG, $P < 0.05$, FDR < 0.1. EGF, epidermal growth factor; FDR, false discovery rate; FGF-2, fibroblast growth factor-2; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IP, interferon-inducible protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

the six women who had detectable HCV RNA at study entry. Each of the associations found in the initial comparison of HIV noncontroller vs. NEG women remained significant with $P$ less than 0.05, though the FDR was more than 0.1 for the associations between IL-12(40) and IL-15 and noncontroller status. In summary, uncontrolled HIV replication was associated with perturbed cytokine and chemokine levels impacting multiple inflammatory and immune pathways.

## Cytokine levels of women on effective HAART are similar but not identical to those in HIV-seronegative women

HAART has been shown to correct much of the inflammation induced by HIV at the level of $CD4^+$ and $CD8^+$ T cells [31]. Abnormalities in a number of serum markers have been shown to improve but not resolve on HAART [32,33], so it was of interest to see whether these changes extended to a broader panel of soluble mediators of inflammation. In general, we found the distortion of the pattern of soluble inflammatory mediators was less pronounced in participants with suppressed viral replication. The extent of increase in TNF-α and decrease in FGF-2 seen among noncontrollers compared with NEG women were mitigated in women in the HAART group. However, the levels of these cytokines did remain different from those in NEG women (Fig. 1 and Table 2). The significant differences compared to NEG women in levels of IP-10, IL-12(p40), and IL-15 that existed in noncontroller participants were not seen in the HAART group. Limitation of analyses to HCV RNA-negative women did not change the associations except that the HAART group had significantly lower IP-10 levels compared to noncontroller women and the FDR was more than 0.1 for FGF-2 in HAART vs. NEG women,

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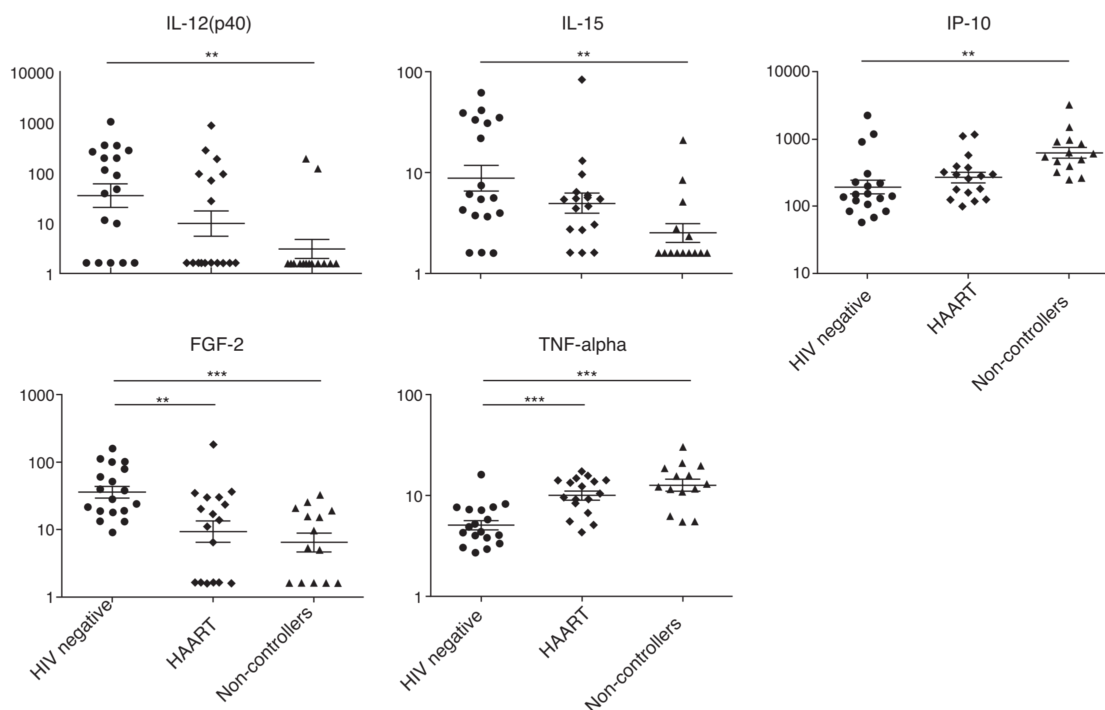


**Fig. 1. Cytokine concentration by clinical group.** Cytokine levels for each of the analytes showing significant differences between groups are shown. Horizontal bar represents mean level, with error bars denoting the standard error of the mean. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. FGF-2, fibroblast growth factor-2; IL, interleukin; IP, interferon-inducible protein; TNF-α, tumor necrosis factor-α.

though the $P$ value remained less than 0.05. These findings demonstrate that although suppression of HIV viremia was associated with much less cytokine perturbation than in the noncontroller women, some cytokines such as TNF-α and FGF-2 did not match levels seen in NEG women.

## Correlation between cytokine levels and plasma viral load

To address the contribution of HIV replication to cytokine levels, we compared analyte levels to plasma HIV RNA copy numbers. Cytokine and plasma viral load levels were log-transformed and linear regression was performed for each analyte. Five analytes were found to be associated with viral load, and of those only IP-10 was also among the five analytes that differed between NEG and noncontroller women ($P < 0.001$). Although IL-12(p40), IL-15, TNF-α, and FGF-2 were all elevated in noncontroller participants, the levels of these cytokines were not associated with viral load (Table 3). IP-10 was the only factor showing a positive correlation with viral load. By contrast, levels of IL-17, MDC, MIP-1β, and fractalkine were inversely related to viral load (Fig. 2a). When HCV RNA-positive women were excluded from the analysis, each of the above correlations remained significant with the exception of MDC, in which the FDR was more than 0.1. Additionally, IL-10 showed a significant positive correlation with viral load and FGF-2 showed a negative correlation after exclusion of HCV RNA-positive women. Irrespective of HCV status, the inclusion of HAART women, whose viral load was set at half the limit of detection of the assay (40 RNA copies/ml), strongly influenced the associations. We, therefore, also performed analyses restricted to the noncontroller women: the only analyte to show a weak positive correlation with the level of viremia was IFN-γ ($r^2 = 0.12$, $P = 0.044$), which was not significant after correction for multiple comparisons. With the exception of IP-10, the cytokine and chemokine levels that were significantly correlated with viral load were distinct from those that differed among the NEG, HAART, and noncontroller groups. Coupled with the finding that levels of TNF-α were elevated and FGF-2 were decreased in the HAART group compared to NEG women (Fig. 1), these findings imply that HIV viremia is not the only determinant factor driving differences in most of the analytes that had distinctive patterns among the NEG, HAART, and noncontroller groups.

## Correlation between cytokine levels and $CD4^+$ T-cell count

The level of viral replication is an important determinant of HIV disease outcome, but even more relevant is the level of $CD4^+$ T cells in the periphery, which is a marker of the degree of immunological impairment. We measured whether cytokine levels correlated with $CD4^+$

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**Table 3. Correlation between cytokine level and HIV viral load.**

| | Slope | $r^2$ | *P* value[a] | FDR |
|---|---|---|---|---|
| **Pro-inflammatory/T cell** | | | | |
| IL-1α | −0.18 | 0.07 | 0.05 | 0.2 |
| IL-1β | −0.02 | 0.003 | 0.7 | 0.9 |
| IL-2 | 0.01 | 0.001 | 0.9 | 1 |
| IL-6 | 0.005 | 0 | 0.9 | 1 |
| IL-7 | 0.01 | 0.005 | 0.6 | 0.9 |
| IL-8 | −0.03 | 0.01 | 0.4 | 0.7 |
| IL-9 | −0.01 | 0.001 | 0.8 | 1 |
| IL-12(p40) | −0.15 | 0.05 | 0.08 | 0.2 |
| IL-12(p70) | −0.12 | 0.07 | 0.04 | 0.2 |
| IL-15 | −0.09 | 0.09 | 0.02 | 0.1 |
| **IL-17** | **−0.17** | **0.13** | **0.005** | **0.04** |
| IFN-γ | 0.06 | 0.01 | 0.4 | 0.8 |
| TNF-α | 0.03 | 0.04 | 0.1 | 0.3 |
| TNF-β | −0.10 | 0.08 | 0.06 | 0.2 |
| GM-CSF | −0.05 | 0.009 | 0.5 | 0.7 |
| **Anti-inflammatory/Th2** | | | | |
| IL-1Rα | 0.02 | 0.001 | 0.8 | 1 |
| sIL-2Rα | −0.13 | 0.03 | 0.2 | 0.4 |
| IL-4 | −0.05 | 0.003 | 0.7 | 0.9 |
| IL-5 | 0.02 | 0.002 | 0.7 | 0.9 |
| IL-10 | 0.08 | 0.08 | 0.03 | 0.1 |
| IL-13 | −0.007 | 0 | 1 | 1 |
| **Chemoattractants** | | | | |
| **IP-10** | **0.14** | **0.26** | **<0.001** | **0.002** |
| MCP-1 | 0.02 | 0.03 | 0.2 | 0.4 |
| MCP-3 | 0.001 | 0 | 1 | 1 |
| **MDC** | **−0.04** | **0.1** | **0.01** | **0.07** |
| MIP-1α | −0.09 | 0.009 | 0.5 | 0.7 |
| **MIP-1β** | **−0.09** | **0.18** | **0.001** | **0.01** |
| Eotaxin | −0.03 | 0.03 | 0.2 | 0.4 |
| **Fractalkine** | **−0.35** | **0.18** | **0.001** | **0.01** |
| **Growth factors** | | | | |
| VEGF | −0.02 | 0.002 | 0.7 | 0.9 |
| EGF | −0.14 | 0.09 | 0.02 | 0.1 |
| FGF-2 | −0.1 | 0.06 | 0.07 | 0.2 |

EGF, epidermal growth factor; FDR, false discovery rate; FGF-2, fibroblast growth factor-2; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IP, interferon-inducible protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.
[a]Analytes shown in bold had significant *P* values after false discovery rate step-down.

T-cell count in HIV-infected women. Two of the five analytes found to be disrupted in noncontroller women also showed a significant correlation with peripheral CD4$^+$ T-cell count, IP-10, and FGF-2 (Table 4, Fig. 2b). In addition, all of the analytes associated with viral load were also significantly inversely associated with CD4$^+$ T-cell count, likely reflecting the predictive value of higher viral load for lower CD4$^+$ T-cell counts. The markers that significantly correlated with both lower viral load and higher CD4$^+$ T-cell count included IL-17, MDC, MIP-1β, and fractalkine. In contrast, IP-10 was associated with higher viral load and lower CD4 cell count. Interestingly, CD4$^+$ T-cell count was the only clinical parameter studied that positively correlated with all three growth factors measured in the panel (VEGF, EGF, and FGF-2). In an attempt to measure whether some factors were associated with CD4$^+$ T-cell count independently of viral load, we performed a second analysis limited to the HAART participants (all with viral load <80 copies/ml). MIP-1β and VEGF correlated with CD4$^+$ T-cell count in the HAART group ($P < 0.05$), though significance was lost after correction for multiple comparisons (data not shown). Finally, none of the correlations between CD4$^+$ T-cell count and soluble markers of inflammation were changed when HCV RNA-positive women were excluded from the analysis.

## Discussion

This study showed that serum levels of the innate immune system pro-inflammatory modulators TNF-α and IP-10 were increased among untreated HIV patients, compared with uninfected comparisons, a finding that is consistent with a broad inflammatory response. Additionally, serum IL-12(p40) and IL-15, important for T-cell homeostasis and function, were decreased in chronic, untreated HIV infection compared to otherwise similar HIV-uninfected women. When compared with untreated women, the HAART recipients showed fewer detectable differences in cytokines from HIV-uninfected persons (two vs. five analytes), though TNF-α and FGF-2 were still different in the HAART compared to NEG group. Finally, although as expected many factors correlated with both plasma viral burden and CD4$^+$ T-cell counts, some factors correlated with one or the other. Interestingly, the growth factors VEGF, EGF, and FGF-2 showed a positive correlation with higher CD4$^+$ T-cell counts.

Analytes found to be elevated in chronic infection in this study have been shown to be elevated using separate test methodologies for TNF-α [19–21] and IP-10 [34]. During primary infection, IP-10 and TNF-α correlated positively with quantitative viral load [35]. We found that the only factor to show a significant positive correlation with viremia in untreated women during chronic HIV infection was IP-10, underlining the importance of this chemokine in the response to HIV and consistent with in-vitro experiments demonstrating its ability to stimulate HIV replication [36]. Elevated IP-10 also has been detected in multiple viral infections, including acute West Nile virus [37], severe influenza infection [38,39], acute HCV [26], and chronic, persistent HCV in this study, suggesting a general role for this chemokine in the immune response to viral infections. Elevated IP-10 levels in chronic HIV infection could be deleterious and contribute to ongoing immune activation and T-cell depletion, supported by the strong negative correlation between IP-10 levels and CD4$^+$ T-cell count we found in this study (Fig. 2b). Finally, the suppression of plasma IL-12 levels in untreated HIV-infected participants is consistent with published work demonstrating cellular

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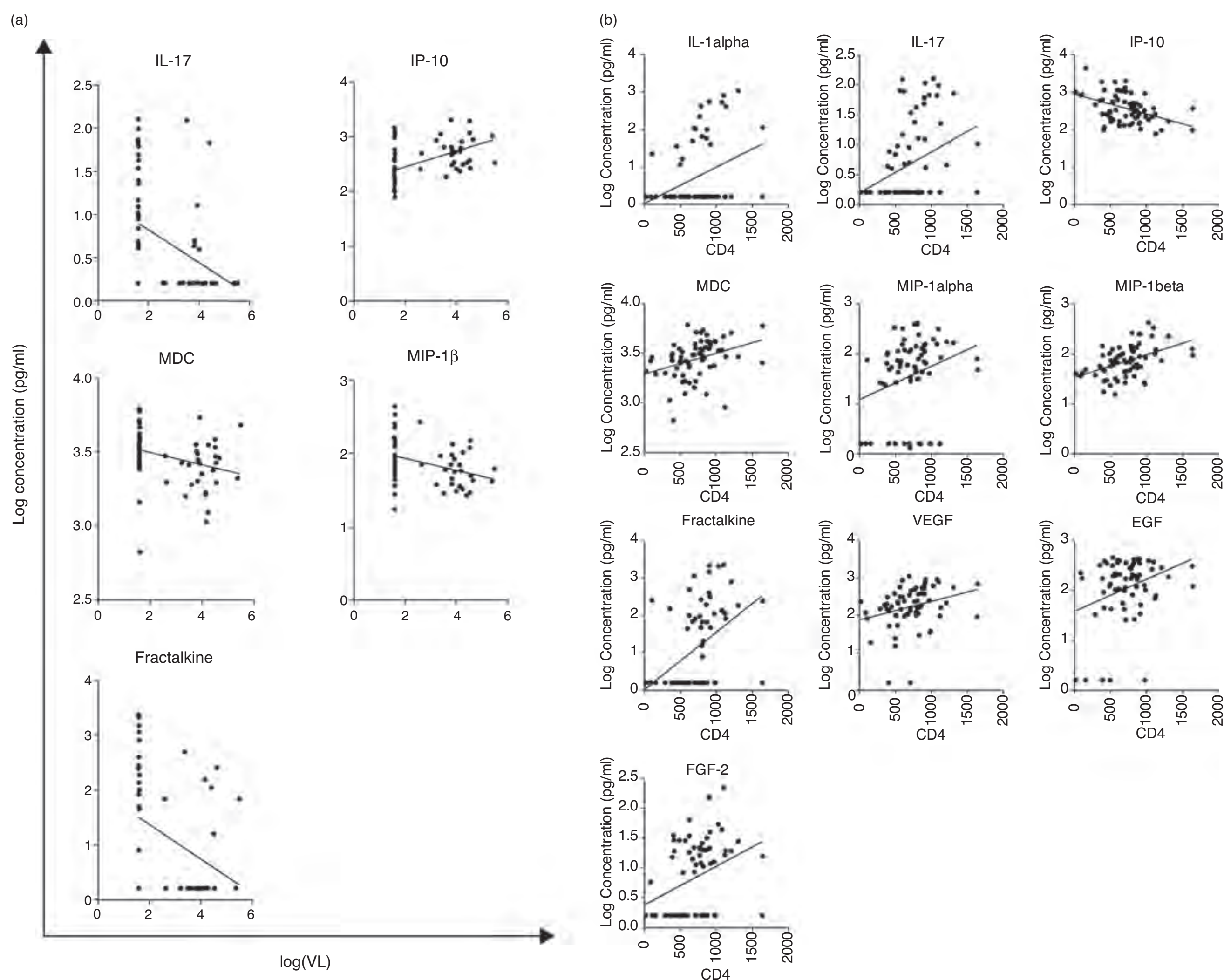


**Fig. 2. Cytokine correlation with HIV plasma viral load and CD4+ T-cell count.** (a) Plots show log(cytokine level) vs. log(viral load) or (b) log(cytokine level) vs. CD4+ T-cell count for each of the analytes showing a significant correlation between the two parameters. A linear regression line is included on each plot. *P* values and $r^2$ values are shown in Tables 3 and 4. FGF-2, fibroblast growth factor-2; IL, interleukin; IP, interferon-inducible protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

defects in production of these cytokines in chronic HIV infection [40,41].

Although most of the cytokine changes previously described to be elevated or reduced during chronic HIV infection were confirmed in our cohort of women with uncontrolled HIV replication, we did not find significant elevations reported by others in predominantly male populations using ELISA tests for IL-6 [42,43], IL-10 [44,45], or FGF-2 [46]. Consistent with prior work, median IL-10 levels in our untreated HIV-infected group were nearly two-fold higher compared to the HIV-negative participants, but this difference was not statistically significant. In contrast, median IL-6 and FGF-2 values were lower in the noncontroller than NEG group. The IL-6 data are consistent with our recent study of acute HIV infection, in which only a subset of participants showed elevations in IL-6 levels [26], though the different assay format (ELISA vs. Luminex) may have been responsible for the discrepant results. We re-tested the samples in the current study using an FGF-2 ELISA kit from the same manufacturer used in the prior study and the significant differences in FGF-2 levels between the clinical groups seen by Luminex testing were not seen using the ELISA kit (data not shown). The discrepancy between the ELISA test and Luminex assay for FGF-2 is potentially due to the different dynamic ranges of the two assays or to the smaller sample size of the current study.

The current study has a number of limitations, including a relatively small sample size, which made detection of only relatively large differences in cytokine concentrations

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**Table 4. Correlation between cytokine level and CD4 cell counts in HIV-positive women.**

| | Slope | $r^2$ | *P* value[a] | FDR |
|---|---|---|---|---|
| **Pro-inflammatory/T cell** | | | | |
| **IL-1α** | **0.001** | **0.14** | **0.004** | **0.02** |
| IL-1β | −0.00007 | 0.004 | 0.6 | 0.8 |
| IL-2 | −0.00005 | 0.001 | 0.8 | 0.9 |
| IL-6 | −0.0001 | 0.004 | 0.7 | 0.8 |
| IL-7 | −0.0002 | 0.04 | 0.2 | 0.3 |
| IL-8 | 0.0002 | 0.01 | 0.4 | 0.6 |
| IL-9 | 0.0003 | 0.02 | 0.4 | 0.6 |
| IL-12(p40) | 0.0006 | 0.04 | 0.1 | 0.3 |
| IL-12(p70) | 0.0004 | 0.03 | 0.2 | 0.3 |
| IL-15 | 0.0003 | 0.05 | 0.1 | 0.3 |
| **IL-17** | **0.0008** | **0.17** | **0.001** | **0.008** |
| IFN-γ | −0.0004 | 0.04 | 0.1 | 0.3 |
| TNF-α | −0.0001 | 0.04 | 0.1 | 0.3 |
| TNF-β | 0.0004 | 0.09 | 0.05 | 0.1 |
| GM-CSF | −0.00001 | 0 | 1 | 1 |
| **Anti-inflammatory/Th2** | | | | |
| IL-1Rα | 0.0001 | 0.002 | 0.8 | 0.9 |
| sIL-2Rα | 0.0002 | 0.007 | 0.6 | 0.8 |
| IL-4 | 0.0002 | 0.002 | 0.7 | 0.9 |
| IL-5 | 0.00001 | 0 | 0.9 | 1 |
| IL-10 | −0.00007 | 0.004 | 0.6 | 0.8 |
| IL-13 | 0.0002 | 0.002 | 0.7 | 0.9 |
| **Chemoattractants** | | | | |
| **IP-10** | **−0.0006** | **0.27** | **<0.001** | **0.001** |
| MCP-1 | −0.00007 | 0.01 | 0.4 | 0.6 |
| MCP-3 | 0.00004 | 0 | 0.9 | 1 |
| **MDC** | **0.0002** | **0.18** | **0.001** | **0.008** |
| **MIP-1α** | **0.0008** | **0.1** | **0.009** | **0.03** |
| **MIP-1β** | **0.0004** | **0.23** | **<0.001** | **0.002** |
| Eotaxin | 0.00009 | 0.01 | 0.4 | 0.6 |
| **Fractalkine** | **0.001** | **0.18** | **0.001** | **0.008** |
| **Growth factors** | | | | |
| **VEGF** | **0.0005** | **0.09** | **0.02** | **0.07** |
| **EGF** | **0.0008** | **0.17** | **0.001** | **0.08** |
| **FGF-2** | **0.0006** | **0.12** | **0.009** | **0.03** |

EGF, epidermal growth factor; FDR, false discovery rate; FGF-2, fibroblast growth factor-2; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; IP, interferon-inducible protein; MDC, macrophage-derived chemokine; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.
[a]Analytes shown in bold had significant *P* values after false discovery rate step-down.

between clinical groups possible. Another potential limitation of the study is the fact that our participants were female and primarily African−American and, therefore, the results may or may not be generalizable to other HIV-infected populations. Data are conflicting regarding the extent to which race affects cytokine responses, with African−Americans showing higher baseline levels of inflammatory cytokines [47], but race having no effect on reactivity of immune cells to IFN-α [48] or on the association between heart failure and inflammatory markers [49]. Finally, we did not formally demonstrate that women initiating HAART showed normalization of perturbed cytokine levels. We also did not test cytokine levels in individuals with high viral load who were taking HAART. However, there are indications from the literature that HAART initiation can normalize or partially normalize inflammatory and coagulation markers [32,33,50]. This, along with the fact that our participants were matched on potential confounders, makes it likely that the differences that we observed between noncontroller and HAART groups were in fact due to HAART.

Recent work by Roberts *et al.* [35] identified a number of factors that were associated with viral load set point and the rate of $CD4^+$ T-cell decline during primary HIV infection. IL-12, IFN-γ, and GM-CSF were associated with lower viral load set point at 12 months and/or slower $CD4^+$ T-cell decline, whereas IL-1α, IL-7, IL-15, and eotaxin showed the opposite effect. In our study, none of these factors except IL-1α showed a correlation with viral load or $CD4^+$ T-cell count, and the association between IL-1α and $CD4^+$ T-cell count in chronic HIV infection was opposite to what was seen in primary infection. These differences highlight the fact that the interaction between HIV and the immune system is likely very different in primary and chronic HIV. For example, biasing the immune system toward a Th1 response with higher IL-12 levels in primary HIV infection may be of benefit to establish a lower viral load set point, whereas after the set point has been reached, many individuals have lost detectable IL-12 secretion (Fig. 1). Similarly, high IP-10 levels during primary HIV infection did not appear to increase subsequent viral load set point or the rate of $CD4^+$ T-cell decline [35], although they were associated with lower $CD4^+$ T-cell counts during chronic HIV infection.

In summary, we found that compared to HIV-negative women, untreated chronic HIV infection was associated with defects in T-cell signaling pathways, coupled with evidence of activation of the innate immune system, and these differences were less apparent or absent in HAART-treated women with undetectable viral load. By examining a broad array of soluble immune mediators, we were able to identify specific analytes that behaved differently from the majority of immune markers. For example, TNF-α and FGF-2 were different in the HAART compared to HIV-uninfected group, as opposed to the majority of other analytes measured. This finding suggests that the pathways driving the dysregulation of these two factors may require very low levels of virus for stimulation (or suppression), are influenced by antiretroviral drugs themselves, or are dependent on immune activation induced by HIV in an indirect fashion. Finally, whereas T-cell homeostasis factors such as IL-2, IL-7, and IL-15 did not appear to be associated with higher $CD4^+$ T-cell counts, the growth factors VEGF, EGF, and FGF-2 were associated with higher $CD4^+$ T-cell counts. Given that the causal role of the relationship is unknown, it is unclear whether these growth factors would have therapeutic potential to ameliorate the immune deficiency associated with HIV infection or would serve as useful biomarkers of a more preserved or restored immune system.

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S.M.K. performed experiments and analyses. S.M.K., E.T.G., R.M.G., S.D., A.L.L., S.J.G., and P.J.N. designed the study. J.Z. and S.W. performed statistical analyses. M.N., M.Y., K.A., H.C., M.H.C., and R.M.G. collected data and provided clinical samples. S.M.K. and P.J.N. wrote the article.

### Conflicts of interest

There are no conflicts of interest.

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