MAJOR ARTICLE

Soluble CD163 Made by Monocyte/Macrophages Is a Novel Marker of HIV Activity in Early and Chronic Infection Prior to and After Anti-retroviral Therapy

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CD163, a monocyte- and macrophage-specific scavenger receptor, is shed during activation as soluble CD163 (sCD163). We have previously demonstrated that monocyte expansion from bone marrow with simian immunodeficiency virus (SIV) infection correlated with plasma sCD163, the rate of AIDS progression, and the severity of macrophage-mediated pathogenesis. Here, we examined sCD163 in human immunodeficiency virus (HIV) infection. sCD163 was elevated in the plasma of individuals with chronic HIV infection (>1 year in duration), compared with HIV-seronegative individuals. With effective antiretroviral therapy (ART), sCD163 levels decreased in parallel with HIV RNA levels but did not return to HIV-seronegative levels, suggesting the presence of residual monocyte/macrophage activation even with plasma viral loads below the limit of detection. In individuals with early HIV infection (≤1 year in duration), effective ART resulted in decreased sCD163 levels that were comparable to levels in HIV-seronegative individuals. sCD163 levels in plasma were positively correlated with the percentage of CD14+CD16+ monocytes and activated CD8+HLA-DR+CD38+ T lymphocytes and were inversely correlated with CD163 expression on CD14+CD16+ monocytes. With ART interruption in subjects with early HIV infection, sCD163 and plasma virus levels spiked but rapidly returned to baseline with reinitiation of ART. This study points to the utility of monocyte- and macrophage-derived sCD163 as a marker of HIV activity that links viral replication with monocyte and macrophage activation. These observations underscore the significance of monocyte and macrophage immune responses with HIV pathogenesis.

Monocytes and macrophages constitute an important component of immune responses against viruses, bridging innate and adaptive immunity [1]. Macrophage activation is pivotal in the pathogenesis of human immunodeficiency virus (HIV) infection, in which the expansion of specific subsets of monocytes and macrophages in blood is observed, which may drive pathogenesis [2, 3].

HIV-mediated destruction of gut mucosal CD4+ T cells and translocation of microbial products into the systemic circulation occurs early in HIV disease [4–6] and potentially activates monocyte and macrophage innate immune responses [7–9]. Uncontrolled hyperreactivity of such pathways can result in chronic immune activation, immune exhaustion, and sepsis [10, 11]. Microbial translocation may be partially responsible for systemic immune activation during AIDS, although monocytes and macrophages are likely sensitized to such LPS stimulation over time [5, 12].

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The hemoglobin scavenger receptor CD163 is expressed exclusively by monocytes and macrophages and preferentially by macrophages of the M2 phenotype [13–17]. Extracellular toll-like receptor (TLR) activation leads to shedding of CD163 as soluble CD163 (sCD163). Because surface CD163 on macrophages functions as an innate immune receptor for bacteria [18], its shedding may be a mechanism to decrease acute and severe monocyte activation and inflammation. Physiological doses of LPS acutely stimulate CD163 shedding within 1 hour, followed by increased expression on the surface of monocytes and macrophages 24 h later [5, 6, 14]. Levels of CD163 on monocytes are inversely correlated with levels of sCD163 found in vitro in tissue culture medium and in vivo in plasma [14, 19, 20]. LPS ligation of TLRs [14, 15], Fcγ receptor cross-linking [21], or mediators of oxidative stress [22] can result in shedding of CD163. CD163 is cleaved by metalloproteinases [22, 23] or the recently described tumor necrosis factor α–converting enzyme (TACE/ADAM17) [24]. In addition, levels of sCD163 in plasma are elevated in macrophage-mediated diseases [16, 25].

Although all CD14+ monocytes express CD163, the CD14+CD16+ population has the highest expression and presumably the highest shedding of CD163 [26]. An expansion of CD14+CD16+ monocytes with HIV infection correlates with histopathological findings and HIV-associated dementia [6, 27–29]. Levels of sCD163 in HIV-infected individuals, the association of sCD163 with disease progression, and the clinical utility of sCD163 in HIV disease management are not defined.

Recently, we demonstrated that monocyte expansion from bone marrow, measured by BrdU expression, correlated with the rate of AIDS progression [2, 29] and the severity of simian immunodeficiency virus (SIV) encephalitis [29]. Monocyte and macrophage expansion, more so than plasma virus and CD4+ T cell counts, correlated with progression to AIDS [2]. The stimulus for such monocyte expansion is not defined; however, the best correlate that we found was sCD163 levels in plasma [29]. Furthermore, CD163-expressing monocytes are elevated during SIV and HIV infection [30, 31]. The correlation between plasma sCD163 and the magnitude of monocyte expansion underscores the importance of innate immune activation in AIDS. Here, we examined sCD163 as a marker of macrophage immune activation in HIV infection.

MATERIAL AND METHODS

Ethics Statement

The patient samples used in this study were obtained from ongoing studies approved by the Institutional Review Boards of the University of California at San Diego (UCSD) and Massachusetts General Hospital (MGH). All study participants and/or legal guardians provided written informed consent.

Individuals With Chronic HIV Infection and HIV-Seronegative Individuals

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated plasma was obtained from 30 individuals with chronic HIV infection (>1 year in duration) at 2 times (Table 1): (1) when a detectable viral load was present, before starting a new regimen, and (2) 3 months after starting a new regimen, when virus levels were undetectable in plasma. Fourteen of the subjects were ART naive, whereas other subjects had previously received a different drug regimen. Plasma samples were also obtained from 29 age-matched HIV-seronegative individuals (HIV-). Hematological parameters were measured at the UCSD Medical Center clinical laboratory.

Subjects With Early HIV Infection

Subjects with early HIV infection are defined as subjects within the first year after seroconversion (as defined by the appearance of HIV-specific antibodies measured by an indeterminate or positive Western blot result [32–34]). All subjects with early HIV infection who were examined were identified during the acute phase of HIV infection (several days or weeks after exposure). Three cohorts of subjects with early HIV infection were examined. Cohort 1 consisted of 14 subjects with early HIV infection examined at 2 times (Table 1): (1) when they were naive to ART and (2) after 3 months of ART. Cohort 2 consisted of 9 subjects with early HIV infection who were examined 1 year after seroconversion, 8 of whom eventually received ART. Cohort 3 consisted of 4 subjects with early HIV infection who underwent planned therapy interruptions. Plasma from EDTA-coagulated blood was obtained and examined for all cohorts. Hematological parameters were measured at the MGH Clinical Microbiology laboratory.

Peripheral Blood Mononuclear Cells (PBMCs)

PBMC samples were only available from 8 of the subjects with chronic HIV infection before ART initiation and from 9 of the subjects with chronic HIV infection and 3 months of ART. Of these samples, 2 were matched pairs of samples obtained before ART initiation and at 3 months after ART initiation. PBMCs were obtained from all 14 subjects with early HIV infection (cohort 1), both before ART and after 3 months of ART. All PBMC samples were obtained at the same time as the plasma samples used to measure sCD163.

Flow Cytometry

Frozen PBMCs were quickly thawed in a 37°C water bath and washed twice in 40 mL of heated RPMI 20% fetal bovine serum. Two million PBMCs were incubated for 15 min with an antibody cocktail of CD14-Pacific blue, CD16-PECy7, HLA-DR-APCCy7, CD163-PerCpCy5.5, CD20-APC, CD3-Alexa Fluor 700, CD38-PE, CD4-FITC, and CD8-Qdot 655. A Live/Dead stain kit was used to exclude nonviable cells. Samples were

Table 1. Demographic and Clinical Characteristics of Study Subjects

Variable	HIV negative	Chronic HIV infection	Early HIV infection
No. of subjects	29	30	14
Age, median years (range)	39 (26–57)	39 (26–57)	39 (22–51)
Male sex, no. (%) of subjects	25 (86)	26 (87)	14 (100)
Ethnicity	4 black, 6 Hispanic, 19 white	5 black, 5 Hispanic, 20 white	2 black, 12 white
Duration of ART[a], median days (range)	NA	97 (75–116)	87 (55–120)
Plasma viral load, $\log_{10}$ copies/mL			
Before ART initiation	NA	4.5 ± 0.9	5.4 ± 1.3
After 3 months of ART	NA	<2.6	<1.7
CD4+ cell count, mean cells/μL ± SD			
Before ART initiation	ND	242 ± 178	481 ± 258
After 3 months of ART	ND	369 ± 199	708 ± 272

NOTE. ART, antiretroviral therapy; HIV, human immunodeficiency virus; NA, not applicable; ND, not determined; SD, standard deviation.

[a] From initiation of ART to the day on which the 3-month ART plasma sample was obtained.

washed and fixed in 1% paraformaldehyde and immediately acquired on a BD FACSAria™.

HIV RNA Quantitation

Different assays to assess plasma viral load were used. In the individuals with long-term HIV infection, either an ultrasensitive (lower limit of detection, 50 [1.7 $\log_{10}$] RNA copies/mL of plasma) or a standard assay (lower limit of detection, 400 (2.6 $\log_{10}$) RNA copies/mL of plasma) (Amplicor Monitor; Roche) was used. Therefore, for all individuals with long-term HIV infection, the lower limit used was 2.6 $\log_{10}$ RNA copies/mL. In the subjects with recent HIV infection, plasma viral load was determined using TaqMan quantitative polymerase chain reaction (PCR) with a lower limit of 48 (1.7 $\log_{10}$) RNA copies/mL of plasma.

Enzyme-Linked Immunosorbent Assay (ELISA) and Limulus Amebocyte Lysate (LAL) Assays

Plasma sCD163, interleukin (IL)-10, and sCD14 were quantified by ELISA according to the manufacturer's protocol (Trillium Diagnostics [sCD163] and R&D Systems [IL-10 and sCD14]). An LAL assay was used to measure LPS as previously described [29]. The lower limit of detection for the LAL assay is 30 pg/mL. Samples from 3 uninfected individuals, 3 individuals with recent HIV infection who had not received ART, and 8 individuals with recent HIV infection and 3 months of ART were below the lower limit of detection of the assay.

Statistical Analysis

Prism software, version 5.0a (GraphPad Software), or Microsoft Excel, version 12.2.4, were used for statistical analysis. An analysis of variance (ANOVA) was first performed for analysis of variation among groups of data. If the ANOVA result was significant ($P < .05$), then post hoc student's t tests were performed. Paired t tests were used for all matched samples. A Spearman rank test was used for all correlations.

Generalized Linear Model-Repeated Measures Analyses

Generalized linear model-repeated measures analyses were used to determine relationships between sCD163, plasma virus, and CD4+ and CD8+ T lymphocyte counts from serial samples in subjects with early HIV infection for which we had multiple samples over time. For each pair of successive measures, the change in viral load, T-cell count, and therapy status (N-N, Y-Y, Y-N, and N-Y) was determined. Generalized linear models were performed using the changes in the aforementioned immunological variables as predictors, subject identifier and therapy status change groups as covariates, and the cross product between the predictor and each of the covariates. Individual effect terms were only reported if the overall model was significant ($P < .05$).

RESULTS

Plasma samples from 30 individuals with chronic HIV infection (at least 1 year since self-reported seropositive HIV test result) were examined at 2 points, before ART and after 3 months of successful ART. sCD163 levels in HIV-infected subjects were compared with levels in age-matched HIV-seronegative individuals (Figure 1A). Parallel to our studies involving individuals with chronic HIV infection, a cohort of 14 HIV-infected subjects who were identified during the acute phase of infection (before seroconversion) or during early infection (within the first year of infection, defined by the appearance of HIV-specific antibodies as identified by an indeterminate or positive Western blot result [32–34]) was examined for sCD163 in plasma samples. Samples were examined during early HIV infection (≤1 year after infection), when patients were naive to ART, and after 3 months of ART (Figure 1A). Plasma sCD163 was significantly different among the groups ($P < .001$ by ANOVA). sCD163 in plasma was elevated in individuals with chronic HIV infection before ART and after 3 months of ART,

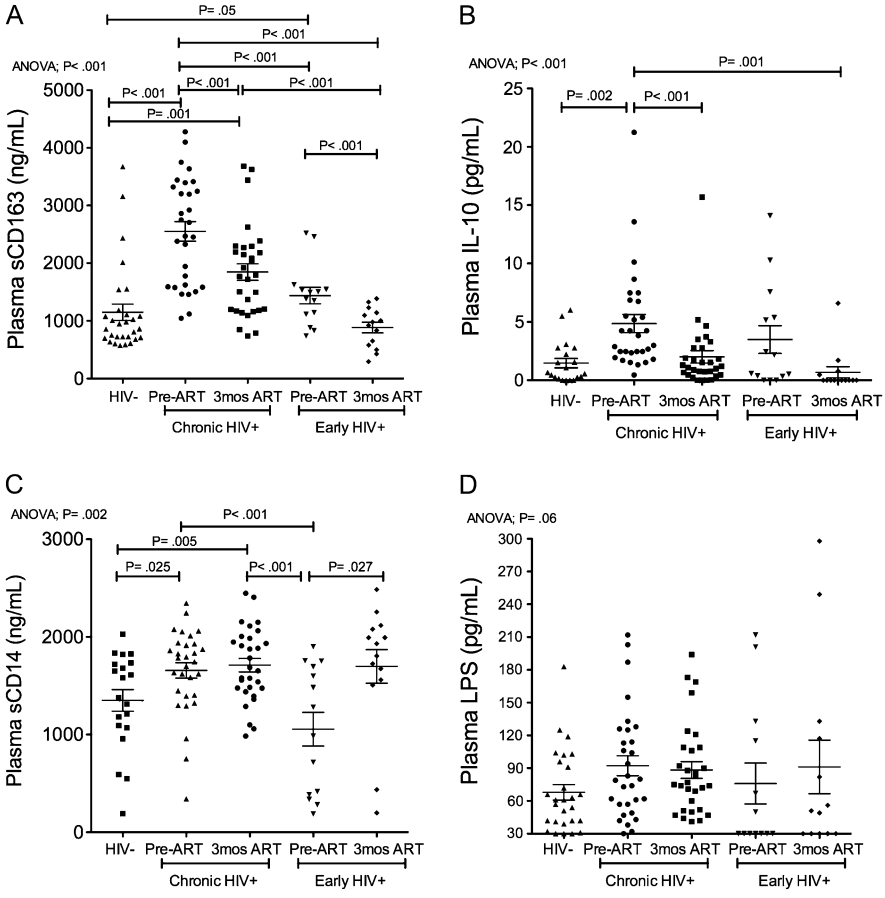

Figure 1. sCD163 levels are significantly increased in plasma during chronic and early human immunodeficiency virus (HIV) infection and are decreased after receipt of antiretroviral therapy (ART). Plasma samples from 30 subjects with chronic HIV infection (Chronic HIV+) and 14 subjects with recent HIV infection (Early HIV+) were examined at 2 time points, as follows: (1) no current ART (Pre-ART) and (2) after 3 months of ART (3mos ART). Samples were compared with samples from 29 age-matched HIV-seronegative individuals (HIV-). Plasma samples were examined for levels of sCD163 (*A*), interleukin (IL)-10 (*B*), sCD14 (*C*), and lipopolysaccharide (*D*). *A*, Plasma sCD163 levels were elevated before ART in both Chronic HIV+ and Early HIV+ groups. After 3 months of ART, sCD163 decreased in both Chronic HIV+ and Early HIV+ groups; however, the levels only returned to levels similar to those in uninfected individuals in the Early HIV+ group. The lines show the mean level and standard error of the mean. An analysis of variance (ANOVA) was used to test for variance between groups. If the ANOVA determined that a difference was statistically significant ($P < .05$), then this was followed by post hoc *t* tests. For comparisons between Chronic HIV+ Pre-ART and Chronic HIV+ 3mos ART and for comparisons between Early HIV+ Pre-ART and Early HIV+ 3mos ART, matched *t* tests were used. Three samples from uninfected individuals, 3 Early HIV+ Pre-ART samples, and 8 Early HIV+ 3mos ART samples had values that were below the lower limit of detection of the Limulus Amebocyte Lysate assay.

compared with HIV-seronegative individuals (Figure 1A; $P < .001$ and $P = .001$, respectively). We found a significant decrease in sCD163 levels in plasma after 3 months of ART in the group with chronic HIV infection (Figure 1A; $P < .001$, by paired *t* test), although sCD163 levels did not return to levels found in HIV-seronegative individuals (Figure 1A), which suggests residual monocyte activation persists even in individuals with undetectable plasma viral loads. Elevated sCD163 levels in plasma are consistent with monocyte activation and stimulation of innate immunity with HIV infection. sCD163 levels in subjects with early HIV infection were higher than those in HIV-seronegative individuals (Figure 1A; $P = .05$) but were lower than in individuals with chronic HIV infection ($P < .001$). After ART initiation, sCD163 levels in individuals with early HIV infection returned to levels seen in HIV-seronegative individuals (Figure 1A; $P = .32$). Examining samples from the same individuals with early HIV infection that were obtained before ART and at 3 months after ART initiation, we found that sCD163 levels decreased with ART that was initiated within the first year after seroconversion (Figure 1A; $P < .001$, by paired *t* test). These data suggest that ART in individuals with recent HIV infection decreases plasma virus and may lower monocyte and macrophage activation, as shown by a decrease in CD163 shedding.

Because IL-10 is an inflammatory mediator that is expressed by monocytes, as well as by many other cell types [35], IL-10 plasma levels were assessed. IL-10 levels were significantly different among groups ($P < .001$, by ANOVA). IL-10 levels were elevated in subjects with chronic HIV infection (before

ART initiation), compared with levels in HIV-seronegative individuals (Figure 1B; $P = .002$). After ART initiation, IL-10 plasma levels decreased significantly (Figure 1B; $P < .001$, by paired t test), to levels similar to those found in HIV seronegative individuals (Figure 1B; $P = .47$). Mean plasma IL-10 levels were similar in early HIV infection and in HIV-seronegative subjects (Figure 1B; $P = .07$). There was no significant change in IL-10 after 3 months of ART (Figure 1B; $P = .06$, by paired t test).

Increased sCD14 and LPS levels in plasma are associated with immune activation in HIV-infected individuals and are possibly associated with HIV pathogenesis [5, 6] and, therefore, were assessed (Figure 1C, D). Plasma sCD14 levels were significantly different among groups ($P = .002$, by ANOVA). sCD14 levels in individuals with chronic HIV infection before and after ART initiation were elevated, compared with levels in HIV-seronegative individuals (Figure 1C; $P = .025$ and $P = .005$, respectively). Interestingly, sCD14 levels in subjects with recent HIV infection before ART initiation were equivalent to those in HIV-seronegative individuals but were significantly elevated after 3 months of ART (Figure 1C; $P = .027$). Plasma LPS levels were not significantly different among groups ($P = .06$, by ANOVA) (Figure 1D). However, there was a trend for higher LPS levels in the patients with chronic infection before ART initiation, compared with levels in HIV-seronegative subjects. Additional factors produced by macrophages, including plasma IL-6 and osteopontin, were not significantly altered with HIV infection or ART (data not shown).

Next, we examined correlations of sCD163 levels with plasma viral load, lymphocyte count, and monocyte count. The percentage change in sCD163 was positively correlated with the percentage change in plasma viral load (Figure 2A; $P = .007$; $r = 0.48$) and was negatively correlated with the percentage change in absolute numbers of CD4+ T lymphocytes (Figure 2B; $P = .02$; $r = -0.42$). As patients improved with ART, sCD163 levels decreased. In patients with chronic HIV infection, monocyte counts correlated with plasma sCD163 levels prior to ART (Figure 2C; $P = .05$; $r = 0.36$) but were not maintained with therapy (Figure 2D; $P = .20$; $r = 0.24$).

We determined whether there was a correlation between sCD163 levels and increased percentage of activated CD14+CD16+ monocytes or CD163 expression on monocytes using PBMCs from a subset of patients with chronic HIV infection (8 before ART and 9 after 3 months of ART, with 2 matched pairs) and all individuals with early HIV infection. PBMC samples and plasma used for the sCD163 assays were obtained simultaneously (Figure 3). As others have shown, we found a trend for a decreased percentage of CD14+CD16+ monocytes in the patients with chronic HIV infection after ART that did not reach significance (Figure 3A) and a significantly decreased percentage of CD14+CD16+ monocytes in individuals with early HIV infection after ART (Figure 3A; $P = .02$, by paired t test). The percentage of CD14+CD16+ monocytes in all HIV-infected patients correlated with plasma sCD163 levels (Figure 3B; overall $r = 0.31$; $P = .04$). When patients with early and chronic infection were examined separately, a significant correlation was found in the population with recent HIV infection but not in the population with chronic infection (Figure 3B; $r = 0.40$; $P = .03$). There were no significant changes in the median fluorescence intensity of CD163 on CD14+CD16+ monocytes after ART (Figure 3C). Interestingly, there was an inverse correlation between CD163 expression on CD14+CD16+ monocytes and sCD163 levels in plasma (Figure 3D; overall $r = -0.45$; $P = .002$), although this negative correlation was not apparent when the patients with early infection and those with chronic infection were examined separately. In individuals with early HIV infection, there were low levels of sCD163 in plasma but high CD163 levels on CD14+CD16+ monocytes. In the individuals with chronic HIV infection, there were high levels of sCD163 in plasma but low CD163 levels on CD14+CD16+ monocytes. These data support the notion that sCD163 is shed from CD14+CD16+ monocytes.

Next, we measured standard markers of immune activation (CD8+HLA-DR+CD38+ T lymphocytes) to assess whether shedding of sCD163 is associated with immune activation (Figure 3E, F). There was a significant decrease in CD8+HLA-DR+CD38+ activated T lymphocytes in the early HIV infection group after ART (Figure 3E; $P < .001$, by paired t test). Similarly, there was a decrease, although not a significant one, in CD8+HLA-DR+CD38+ activated T lymphocytes in the individuals with chronic HIV infection after ART (Figure 3E; $P = .15$). Overall, sCD163 significantly correlated with the percentage of CD8+HLA-DR+CD38+ T lymphocytes (Figure 3F; overall $r = 0.30$; $P = .04$). Within groups, sCD163 significantly correlated with the percentage of CD8+HLA-DR+CD38+ T lymphocytes in both subjects with early infection (Figure 3F; $r = 0.45$; $P = .02$) and subjects with chronic infection (Figure 3F; $r = 0.61$; $P = .01$). Thus, shedding of sCD163 is strongly associated with immune activation.

In light of data obtained from individuals with early HIV infection before and after 3 months of ART, we studied an additional cohort of 9 subjects with recent HIV infection who were followed-up for the entire first year after seroconversion. Longitudinal analyses of sCD163, determination of plasma viral load, and determination of absolute numbers of CD8+ and CD4+ T lymphocytes were performed (Figure 4A, B). sCD163 level was elevated at seroconversion and readily decreased following ART initiation (Figure 4A, B). Generalized linear model repeated-measures analyses were used to analyze relationships between sCD163 level, plasma viral load, and number of CD4+ and CD8+ T lymphocytes from serial samples in subjects with recent HIV infection. Changes in plasma viral load predicted changes in sCD163 level ($P = .03$). A relationship was seen between sCD163 level and CD8+ cell count ($P < .001$), but not

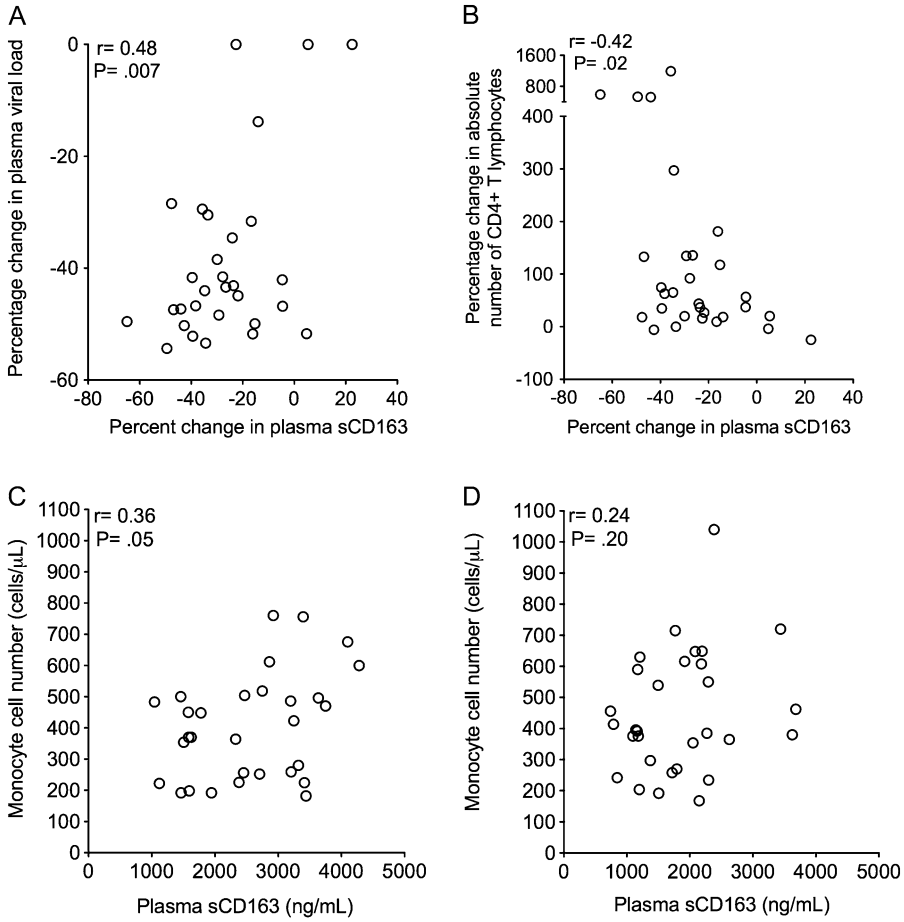

Figure 2. Percentage change in plasma viral load and absolute number of CD4+ T lymphocytes correlated with change in plasma sCD163 levels after antiretroviral therapy (ART). To directly measure the effect of ART on virological parameters examined in individuals with chronic human immunodeficiency virus (HIV) infection, the percentage change was calculated ([{value before ART – value after 3 months of ART}/value before ART] $\times$100%), where a negative percentage represented a decrease and a positive percentage represented an increase after 3 months of ART. The percentage change in sCD163 level was correlated with the percentage change in plasma virus (*A*) and absolute number of CD4+ T lymphocytes (*B*). The absolute number of monocytes was correlated to sCD163 levels in plasma samples from subjects with chronic HIV infection prior to ART (*C*) but not after 3 months of ART (*D*). r = Spearman's correlation coefficient. P value of $\leq$ .05 is statistically significant.

CD4+ cell count. Four individuals with recent HIV infection underwent planned interruptions in therapy after the first year of infection. During interruption of therapy (Figure 4C, D), plasma virus was detectable, followed by increased sCD163 levels and CD8+ T lymphocyte counts (Figure 4C, D). With reinitiation of ART, plasma viral load, sCD163 level, and CD8+ T-cell count decreased to levels similar to those before ART interruption (Figure 4C, D). The number of CD4+ T lymphocytes was not altered (Figure 4C, D). These data underscore the role of macrophage activation and enhanced innate immunity during periods of interrupted therapy, which can be monitored by sCD163 level in plasma.

DISCUSSION

Here, we report that levels of sCD163, a surface marker shed by activated monocytes and macrophages, paralleled levels of HIV RNA prior to and after ART in both individuals with early HIV infection and individuals with chronic HIV infection. In individuals with early HIV infection, successful ART results in the return of sCD163 levels to baseline levels that are equivalent to those in HIV-seronegative individuals; this is in contrast to successful ART in individuals with chronic HIV infection. Additionally, sCD163 levels in plasma correlated with the expansion of CD14+CD16+ monocytes, a decrease in CD163 surface expression, and an increased percentage of activated CD8+HLA-DR+CD38+ T lymphocytes. We have previously demonstrated that sCD163 levels in plasma correlated with the expansion of monocytes and macrophages from bone marrow, rapid onset of SIV AIDS, and central nervous system neuropathology [2, 29]. Overall, these results suggest that sCD163 is a novel marker of HIV activity and likely macrophage-mediated disease progression associated with HIV infection.

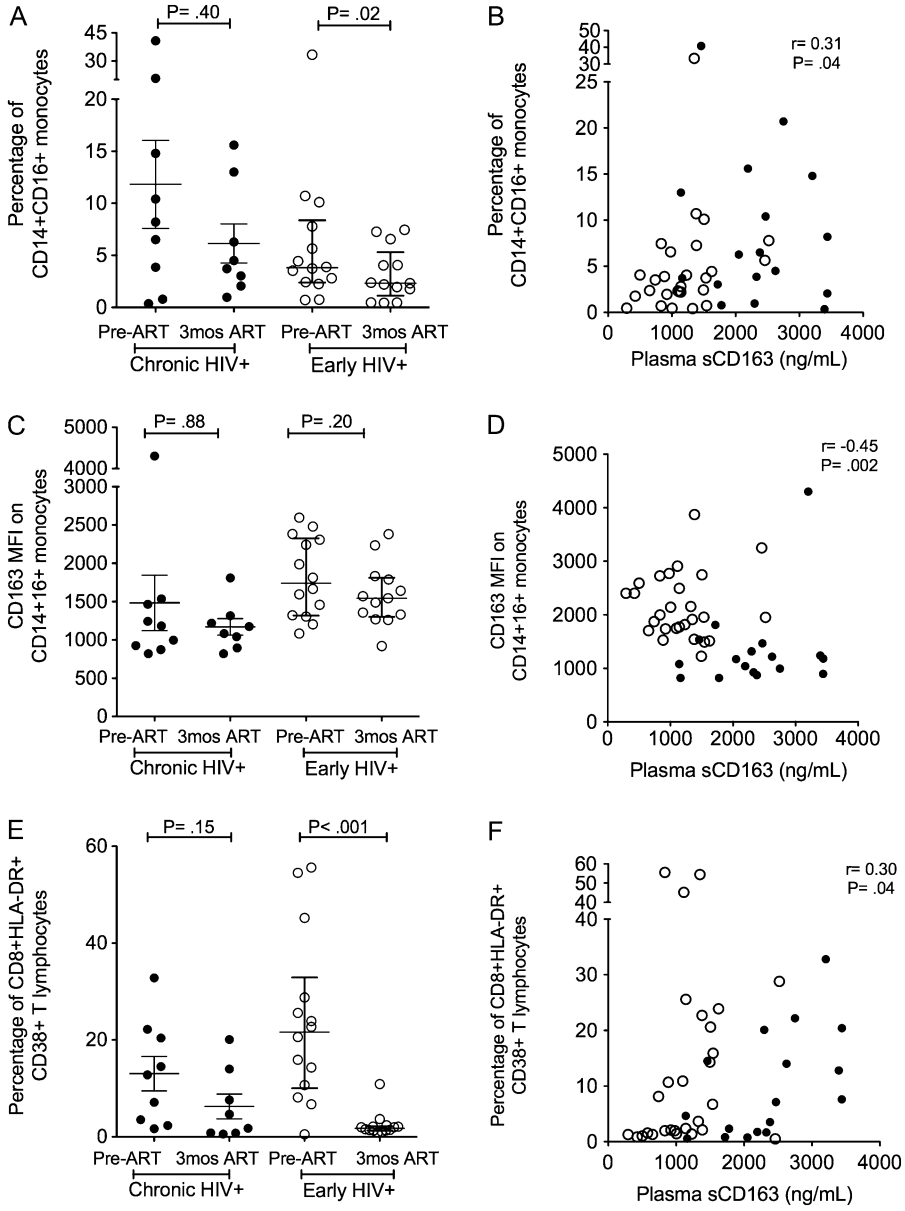

Figure 3. The percentage of CD14+CD16+ monocytes and CD8+HLA-DR+CD38+ T lymphocytes correlates with sCD163. Peripheral blood mononuclear cells (PBMCs) from 8 patients with chronic human immunodeficiency virus (HIV) infection (Chronic HIV+) before antiretroviral therapy (Pre-ART) (*closed circles*) and 9 patients after 3 months of antiretroviral therapy (3mos ART) (2 sets of samples were from matched patients) as well as PBMCs from all 14 HIV-infected individuals with early infection (Early HIV+) (*open circles*) obtained Pre-ART and 3mos ART were examined. Flow cytometry analysis was used to determine the percentage of CD14+CD16+ monocytes (*A, B*), the median fluorescence intensity (MFI) of CD163 on CD14+CD16+ monocytes (*C, D*), and the percentage of activated CD8+HLA-DR+CD38+ T lymphocytes (*E, F*) in all PBMC samples. In *A, C,* and *E,* nonparametric Mann-Whitney *t* tests were used where a *P* value of $\leq$ .05 is significant. For the Early HIV+ samples, paired *t* tests were used, because all samples were matched Pre-ART and 3mos ART. In *B, D,* and *F,* the correlations were performed with both Early HIV+ samples (*open circles*) and Chronic HIV+ samples (*closed circles*). *B,* the percentage of CD14+CD16+ monocytes significantly correlated to plasma sCD163. *D,* the expression level of CD163 on CD14+CD16+ monocytes inversely correlated to plasma sCD163. *F,* the percentage of activated CD8+HLA-DR+CD38+ T lymphocytes significantly correlated to plasma sCD163 level. The *r* and *P* values shown are for the overall correlation, including both patients with recent infection and patients with chronic infection. r = Spearman's correlation coefficient, *P* value of $\leq$ .05 is significant.

To date, 2 reports have described CD163 shedding associated with HIV infection [36, 37]. One case report described a patient with impaired hemoglobin scavenging following acute HIV infection [37]. The patient had low levels of CD163 on monocytes and accumulation of haptoglobin:hemoglobin in the serum, but the patient's condition improved with ART, which suggests a transient CD163 impairment that was mediated by HIV [37]. The second report showed that sCD163 levels in

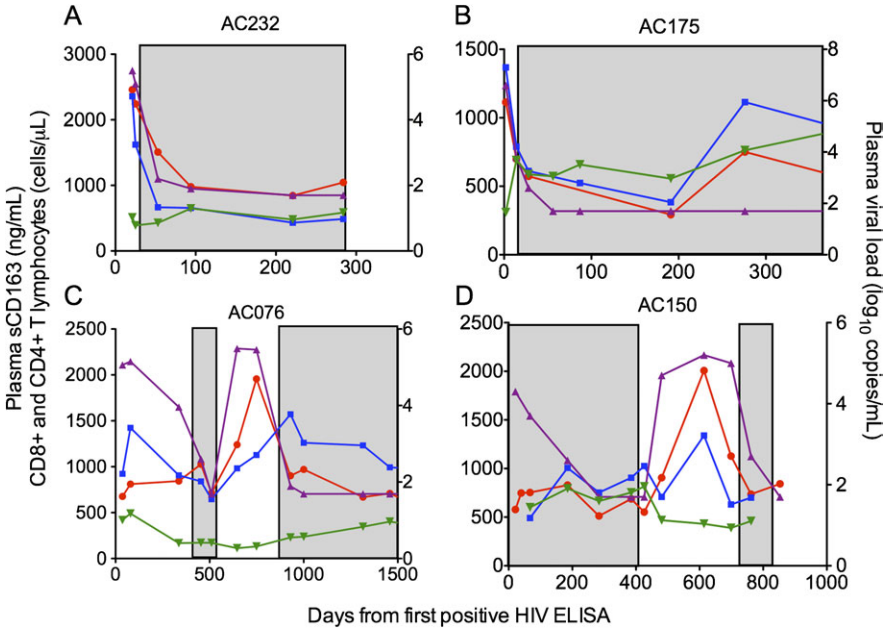

Figure 4. Plasma viral load and CD8+ T lymphocyte count, but not CD4+ T lymphocyte count, parallel sCD163 levels in plasma, even during antiretroviral therapy (ART) interruption. Plasma samples were examined for sCD163 (*red; left axis*), plasma viral load (*purple; right axis*), absolute number of CD8+ T lymphocytes (*blue; left axis*), and absolute number of CD4+ T lymphocytes (*green; left axis*). The shaded gray area represents samples from patients receiving ART, and the white area represents samples from patients with no ART. Shown are data for 2 representative individuals (*A, B*) with early acquired human immunodeficiency virus (HIV) infection of 9 individuals who were examined. Samples were plotted over the first year of infection from date of seroconversion (seroconversion was defined as the day of the first positive Western blot result). Plasma viral load and CD8+ lymphocyte count paralleled sCD163 levels in plasma. Also shown are data for 2 representative individuals (*C, D*) with early HIV infection of 4 individuals who were examined during periods of ART interruption and reinitiation. sCD163 levels increased during therapy interruption, paralleling plasma viral load and absolute numbers of CD8+ T lymphocytes, and decreased upon reinitiation of ART. CD4+ T lymphocyte counts were not affected by therapy interruption.

serum predicted survival in patients with tuberculosis (TB) and that those with HIV infection and TB had the highest levels of sCD163 [36]. The relationship between HIV, monocytes and macrophages, and sCD163 was less clear in this study because the patients had TB coinfection [36].

We demonstrate that sCD163 levels in plasma return to normal levels after administration of ART in patients with early HIV infection but not in patients with chronic HIV infection. These data suggest that chronic macrophage activation and potential dysfunction are not immediate outcomes of HIV infection. Instead, our study indicates that it appears to take at least 1 year to attain chronic macrophage activation states, as defined by an elevation in sCD163 levels, which is incompletely responsive to suppressive ART.

HIV-infected monocytes are found in individuals receiving ART, and proviral DNA can be measured in monocytes despite undetectable plasma virus levels [38–40]. These data, along with the observation that sCD163 levels returned to levels found in HIV-seronegative individuals after successful ART in individuals with recent HIV infection, argues that early ART may dampen inappropriately activated immune responses. In individuals with chronic infection, there remained residual macrophage activation, as seen by sCD163 levels that were higher than those in seronegative controls. If this is true, this might suggest that sCD163 is a marker of HIV-disease activity in longer-lived cells, such as macrophages.

Health care providers usually withhold ART until CD4+ T cell counts decrease below 350 cells/μL. This study suggests that waiting until such thresholds are reached may be detrimental, because ART initiation in subjects with early infection normalized monocyte activation, unlike in patients with chronic infection. Such irreversible monocyte activation can have adverse consequences in individuals with HIV infection, including insulin resistance, lipodystrophy, and vascular complications. We note that, in clinical practice, ART is frequently unsuccessful. Our data would predict that, with unsuccessful ART, sCD163 levels would remain elevated. It is possible that the changes in sCD163 levels that we found could be a direct effect of antiretrovirals on macrophages, although we believe it is more likely that antiretrovirals act indirectly through reducing viral replication. Of the standard antiretrovirals in clinical use, few have demonstrated direct immunosuppressive effects on macrophages or T lymphocytes [41, 42], and they are thus unlikely to account for the sCD163 modulation effects that we describe.

We showed a significant correlation between sCD163 levels in plasma and percentage of CD14+CD16+ monocytes and

activated CD8+HLA-DR+CD38+ T lymphocytes in HIV-infectedsubjects. Expansion of CD14+CD16+ monocytes corresponds with disease progression in AIDS, including HIV encephalitis, and their abundance is indicative of monocyte activation [27, 43, 44]. CD14+CD16+ monocytes have the highest levels of CD163 surface expression [26], suggesting that sCD163 shed during HIV infection is likely from CD14+CD16+ monocytes. Additionally, it is possible that sCD163 in plasma also comes from activated tissue macrophages [29]. Here, we show that sCD163 levels in plasma are inversely correlated with CD163 expression on CD14+CD16+ monocytes. This increase in the percentage of CD14+CD16+ monocytes, as well as decreased CD163 expression on monocytes, correlated with sCD163 levels in plasma.

The quantity of LPS that we detected in the plasma of HIV-infected and HIV-seronegative subjects was similar to data published in past studies [5, 6, 45, 46]. However, we did not find a statistically significant difference among HIV-infected groups. Differences may be attributable to different LAL assays used, differences in the patient populations studied or in the number of patients examined, and/or macrophage tolerance to LPS in individuals with long-term infection.

Exact functions of sCD163 are unknown, but it is involved in recycling extracellular iron and thus in inhibiting the growth of bacterial pathogens [14, 47]. Additionally, sCD163 has been shown to directly inhibit T lymphocyte activation and proliferation [48]. Early in HIV infection, HIV is generally macrophage-tropic (M-tropic), and effective ART decreases sCD163 levels to levels that are similar to those in control subjects. As a natural inhibitor of T-cell activation, sCD163 increases may represent an attempt of anti-inflammatory macrophages to limit viral replication and spread. Later in the course of disease, the sCD163 response is less effective and, with the inability of ART to restore sCD163 to normal levels, may signify a switch from M-tropic to T-cell–tropic HIV. The enhanced sCD163 levels in the individuals with long-term HIV infection may signify the presence of a chronic macrophage activation state attempting to control T-tropic HIV infection through inhibiting T-cell activation and proliferation.

Here, we have identified the presence of the protein marker sCD163, which is made by monocytes and macrophages and is linked to monocyte expansion. Because monocyte expansion predicts progression to AIDS [2, 27, 29], sCD163 levels may be useful in predicting HIV disease progression. This is the first observation within HIV-infected individuals of a marker in plasma that is both exclusive to monocytes and a marker of activation of the innate immune system that parallels HIV replication. These results underscore the significance of innate immune responses by monocytes and macrophages in HIV pathogenesis. This work points to the usefulness of sCD163 levels as a marker of HIV activity for AIDS therapies targeting HIV replication, monocyte and macrophage immune activation, and pathogenesis.

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