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Normalization of Soluble CD163 Levels After Institution of Antiretroviral Therapy During Acute HIV Infection Tracks with Fewer Neurological Abnormalities

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Background. Myeloid activation contributes to cognitive impairment in chronic human immunodeficiency virus (HIV) infection. We explored whether combination antiretroviral therapy (cART) initiation during acute HIV infection impacts CD163 shedding, a myeloid activation marker, and in turn, implications on the central nervous system (CNS).

Methods. We measured soluble CD163 (sCD163) levels in plasma and cerebrospinal fluid (CSF) by enzyme-linked immunosorbent assay in Thais who initiated cART during acute HIV infection (Fiebig stages I–IV). Examination of CNS involvement included neuropsychological testing and analysis of brain metabolites by magnetic resonance spectroscopy. Chronic HIV-infected or uninfected Thais served as controls.

Results. We examined 51 adults with acute HIV infection (Fiebig stages I–III; male sex, >90%; age, 31 years). sCD163 levels before and after cART in Fiebig stage I/II were comparable to those in uninfected controls (plasma levels, 97.9 and 93.6 ng/mL, respectively, vs 99.5 ng/mL; CSF levels, 6.7 and 6.4 ng/mL, respectively, vs 7.1 ng/mL). In Fiebig stage III, sCD163 levels were elevated before cART as compared to those in uninfected controls (plasma levels, 135 ng/mL; CSF levels, 10 ng/mL; $P < .01$ for both comparisons) before normalization after cART (plasma levels, 90.1 ng/mL; CSF levels, 6.5 ng/mL). Before cART, higher sCD163 levels during Fiebig stage III correlated with poor CNS measures (eg, decreased N-acetylaspartate levels), but paradoxically, during Fiebig stage I/II, this association was linked with favorable CNS outcomes (eg, higher neuropsychological test scores). After cART initiation, higher sCD163 levels during Fiebig stage III were associated with negative CNS indices (eg, worse neuropsychological test scores).

Conclusion. Initiation of cART early during acute HIV infection (ie, during Fiebig stage I/II) may decrease inflammation, preventing shedding of CD163, which in turn might lower the risk of brain injury.

Keywords. Soluble CD163; acute HIV infection; neurocognitive impairment; central nervous system; cerebrospinal fluid; plasma; combination antiretroviral therapy.

Cognitive impairment persists in 30%–50% of individuals with chronic human immunodeficiency virus (HIV) infection despite access to combination antiretroviral therapy (cART) [1, 2]. In this cognitive impairment setting, indices of inflammation, such as plasma interferon α, interleukin 6, and soluble interleukin 2 receptor levels, are elevated in individuals with worsening HIV-associated neurocognitive disorder (HAND), compared with unimpaired persons or individuals with stable HAND diagnoses [3]. In Thais with chronic HIV infection, peripheral blood mononuclear cells (PBMCs) enriched for $CD14^+$ cells from individuals with persistent HAND secrete greater levels of CXCL8 (also known as interleukin 8) and CCL2 (also known as monocyte chemoattractant protein 1) as compared to individuals with normal cognition [4]. These data highlight the crucial role of chronic inflammation in the context of HAND even during plasma viral suppression. Recent studies have highlighted the benefits of cART initiated during acute HIV infection in reducing select parameters of inflammation [5, 6]. Whether early cART initiation during acute HIV infection influences CD163 shedding is unknown.

Plasma sCD163, shed after monocyte/macrophage activation [7, 8], is an independent predictor of all-cause mortality

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during HIV infection [9] and a biomarker in the context of cognitive impairment. In cART-treated, chronically HIV-infected individuals with mild neurocognitive disorder, plasma sCD163 levels are elevated, compared to persons who are cognitively normal or who have asymptomatic neurocognitive impairment [10]. Similarly, in the Women's Interagency HIV Study, higher plasma sCD163 levels are associated with worse overall cognitive performance and worse performance within multiple subdomains [11]. Plasma sCD163 levels also correlate with cerebrospinal fluid (CSF) levels of neopterin, an indicator of monocyte activation for which levels are elevated during HIV infection [12, 13], and to postmortem markers of synaptodendritic damage and microgliosis [14].

The impact of early cART on adverse non-AIDS events, such as cognitive function, in acute HIV infection is still unclear. Here, we evaluated plasma and CSF sCD163 levels and the relationship to central nervous system (CNS) outcomes in a prospective study of cART initiation during acute HIV infection.

METHODS

Cohort Descriptions

Acute HIV Infection (RV254/SEARCH010) Cohort

Walk-in clients seeking volunteer counseling and testing at the Thai Red Cross Anonymous Clinic were screened for acute HIV infection by pooled nucleic acid testing and a fourth-generation immunoassay (clinical trials registration NCT00796146) [15]. Participants were subsequently categorized as Fiebig stage I–IV [16, 17]. Participants were offered immediate cART via a local protocol (clinical trials registration NCT00796263). Our analyses included 9 individuals in Fiebig stage I, 10 in Fiebig stage II, and 32 in Fiebig stage III. Excluding the participant who did not begin cART, 86% of participants initiated cART within 3 days of study entry (median, 1.5 days; range, 0–5 days).

Control Cohorts

Fifty-three chronically HIV-infected participants who were cART naive and met Thai Ministry of Health guidelines to initiate therapy were enrolled in SEARCH011 (clinical trials registration NCT00782808), as previously described [13, 17]. Eighteen demographically matched uninfected individuals from Thailand were also evaluated as controls (SEARCH013; clinical trials registration NCT01397669).

Measurement of Soluble Levels of Monocyte/Macrophage Activation Markers by Enzyme-Linked Immunosorbent Assays (ELISAs)

sCD163 and neopterin levels were measured by single-plex ELISAs (Biorad [Hercules, CA] and Genway [San Diego, CA], respectively) in plasma and CSF samples collected before and 24 and 48 weeks after starting treatment, among individuals with acute HIV infection, or 48 weeks after treatment initiation, among those with chronic HIV infection.

Neurocognitive Assessment by Neuropsychological Testing

Acute HIV Infection and Uninfected Controls

Neuropsychological tests included Trail Making A, Color Trails 1 and 2, and Grooved Pegboard (nondominant hand) to compute a summary neuropsychological *z*-4 score. All raw scores were transformed to standard *z* scores using Thai normative data.

Chronic HIV Infection

Study participants completed a 1-hour neuropsychological testing battery, as previously described [18, 19]. All raw scores were transformed to standard *z* scores using Thai normative data. A measure of global performance (neuropsychological *z* global score) was calculated as the arithmetic mean of all tests with domain scores calculated from domain-specific components of the battery.

Assessment of Brain Metabolites by Magnetic Resonance Spectroscopy (MRS)

Single-voxel proton MRS scanning was performed for all HIV-infected participants using a 1.5 T GE scanner located at Chulalongkorn Hospital, as previously described [20, 21]. Assessment of alterations in the following brain metabolite levels during HIV infection were assessed [20, 22–26]: a combination of N-acetylaspartate and N-acetylaspartylglutamic acid, myoinositol, choline, a combination of glutamate and glutamine, and creatine, before and after cART initiation (24 weeks for acute HIV infection and 48 weeks for chronic HIV infection). Brain metabolite levels were measured in 4 brain regions reported to have abnormal brain chemical profiles in HIV: basal ganglia, frontal white matter, frontal gray matter, and posterior cingulate gyrus [20, 23, 24, 27–29].

CD163 Cell Surface Expression by Flow Cytometry

In participants with available cryopreserved PBMCs, specimens were thawed and stained with viability dye (yellow Live/Dead Fixable Dead Cell Stain), followed by staining with monoclonal antibodies to exclude lymphocytes (CD3-V500), B cells (CD19-PE-Cy7 and CD20-PE-Cy7), and natural killer cells (CD56-PE-Cy7). Monocytes, positive for HLA-DR (APC-H7), were then classified into the following subsets on the basis of CD14 (Qdot605), CD16 (AlexaFluor700) expression: classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$), or nonclassical ($CD14^{+}CD16^{+}$) monocytes. Monocyte populations were further assessed for CD163 expression (Alexa Fluor 488). Cells were fixed with 1% paraformaldehyde and acquired on a custom 4-laser LSR Fortessa (BD Biosciences, San Jose, CA). Compensation and gating analyses were performed using FlowJo (Treestar, Ashland, OR). All monoclonal antibodies were purchased from BD Biosciences, except for CD14–Qdot 605 and Live/Dead Stain (Life Technologies, Grand Island, NY) and CD163–Alexa Fluor 488 (R&D Systems, Minneapolis, MN). The gating strategy for identification of monocytes, their

subsets, and receptor expression are shown in Supplementary Figure 1.

Statistical Analyses

Demographic and clinical characteristics are presented using median and interquartile ranges, except for sex, which is given as a percentage. Comparisons between continuous variables were examined using Kruskal-Wallis tests, and those between categorical variables were examined using χ^2 tests. For CD163 outcomes, Wilcoxon rank sum tests were conducted to compare between groups, and Friedman and Wilcoxon signed rank tests were used to compare within groups over time, without adjustment for multiple comparisons. Associations between 2 continuous variables were evaluated by Spearman correlation. Multiple linear regression analyses were performed to explore the relationship between circulating sCD163 and cell-surface CD163 levels and Fiebig stages with CNS measures. Statistical analyses were performed using R, version 3.2.2, or GraphPad Prism, version 7 (GraphPad Software, San Diego, CA).

RESULTS

Participant Characteristics

Clinical and demographic characteristics of the participants are shown in Table 1. The overall median age was 33 years for the uninfected group, 31 and 28 years for Fiebig stage I/II and III acute HIV infection, respectively, and 34 years for the chronically HIV-infected cohort. A total of 50% of uninfected controls, 95% and 91% of Fiebig stage I/II and III, respectively, and 45% of participants with chronic HIV infection were male. Acute HIV-infected participants were randomized to receive either a cART regimen, consisting of tenofovir, emtricitabine, and efavirenz, or the 3-drug cART regimen plus maraviroc and raltegravir (cART+) during the first 24 weeks. A total of 47% and 28% in Fiebig stage I/II and III, respectively, received cART, and 47% and 72%, respectively, received cART+. One participant in Fiebig stage I/II did not initiate cART. Chronically HIV-infected participants received a cART regimen that included lamivudine, nevirapine, and either stavudine, zidovudine, or tenofovir. Treatment for participants intolerant to this regimen was modified on the basis of clinical acumen [30].

Comparison of Plasma and CSF sCD163 Levels in Acute and Chronic HIV Infection

Before cART, median plasma sCD163 levels in participants in Fiebig stage I/II (97.9 ng/mL) were lower than in participants in Fiebig stage III (134.8 ng/mL; $P = .002$) or chronic HIV infection (582.9 ng/mL; $P < .0001$) and were similar to uninfected controls (99.5 ng/mL; Figure 1A). After cART initiation in participants in Fiebig stage I/II, plasma sCD163 levels transiently increased at week 24 (to 109.0 ng/mL; $P = .034$) and by week 48 decreased to pre-cART levels (93.6 ng/mL; Figure 1A). After cART initiation in participants in Fiebig stage III, plasma sCD163 levels continuously declined (to 109.9 ng/mL at week 24; $P = .003$), and by week 48 (90.1 ng/mL; $P < .0001$) there were no differences compared to uninfected levels (Figure 1A). After 48 weeks of cART, plasma sCD163 levels in participants with chronic HIV infection decreased (to 271.2 ng/mL) but remained elevated as compared to participants in acute HIV infection and uninfected controls ($P < .0001$ for all comparisons; Figure 1A). Examination of the longitudinal data from the cohorts revealed declines in plasma sCD163 levels during Fiebig stage III and chronic HIV infection after cART ($P < .001$ for all comparisons). In participants in Fiebig stage I/II, there was a difference between time points ($P = .047$), but plasma sCD163 levels never differed from uninfected controls. For the longitudinal analyses, Friedman and Wilcoxon signed rank tests were performed, without adjustment for multiple comparisons.

Before cART, CSF sCD163 levels in participants in Fiebig stage I/II (6.7 ng/mL) were similar to uninfected controls (7.1 ng/mL) and did not change after cART (6.4 ng/mL; Figure 1B). However, pre-cART CSF sCD163 levels were elevated in participants in Fiebig stage III (10.0 ng/mL) and chronic HIV infection (9.53 ng/mL), compared with participants in Fiebig stage I/II ($P = .008$ and $P = .017$, respectively) or uninfected controls

Table 1. Clinical and Demographic Characteristics

Characteristic	HIV Uninfected (n = 18)	HIV Infected			P[a]
		Acute (Fiebig I/II) (n = 19)	Acute (Fiebig III) (n = 32)	Chronic (n = 53)	
Male sex, no. (%)	9 (50)	18 (95)	29 (91)	24 (45)	<.0001
Age, y	33 (28–39)	31 (26–39)	28 (23–29)	34 (29–37)	<.001
Education duration, y	11 (7–14)	18 (16–20)	17 (14–18)	11 (8–14)	<.0001
NPZ global score	0.23 (−0.13–0.86)	−0.09 (−0.44–0.17)	−0.22 (−0.49–0.07)	−0.06 (−0.58–0.31)	.863
Log10 plasma viral load, copies/mL	…	5.08 (4.28–5.54)	5.92 (5.52–6.88)	4.85 (4.48–5.50)	<.0001
CD4+ T-cell count, cells/μL	…	447 (307–567)	370 (293–470)	228 (114–353)	<.0001
CD8+ T-cell count, cells/μL	…	271 (228–396)	560 (426–999)	701 (559–1025)	<.0001

Data are presented as median (interquartile range), unless otherwise indicated.

Abbreviation: NPZ, neuropsychological *z* test.

[a]By the χ^2 test (for categorical variables) and the Kruskal-Wallis test (for continuous variables), for comparison of the 3 HIV-positive groups.

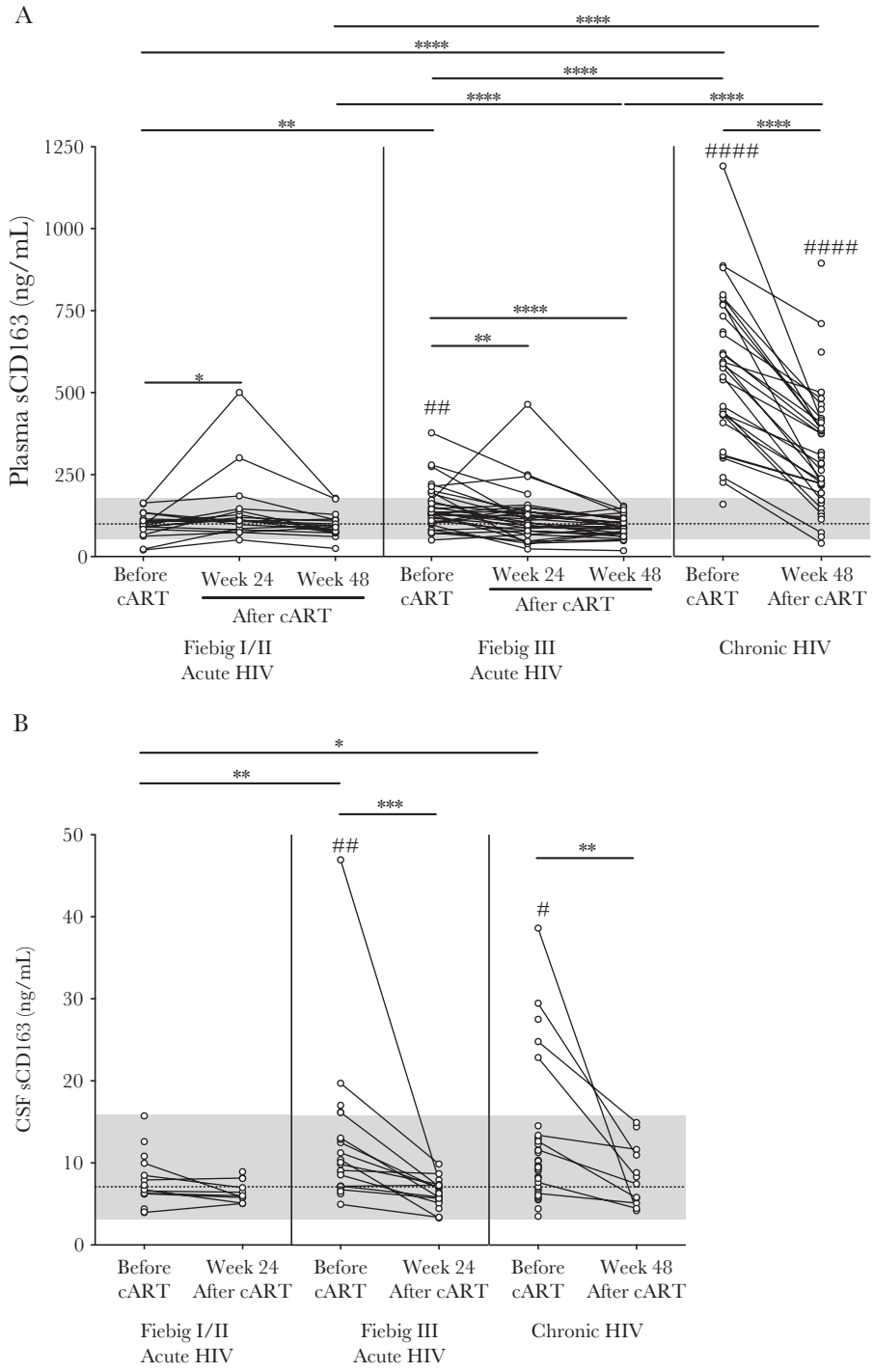

Figure 1. Plasma (*A*) and cerebrospinal fluid (CSF; *B*) soluble CD163 (sCD163) levels during acute human immunodeficiency virus (HIV) infection, broken down by Fiebig stage, and chronic HIV infection, before and 24 and/or 48 weeks after combination antiretroviral therapy (cART) initiation. Data points that are connected represent longitudinal data for 1 participant. Dashed lines indicate uninfected control median levels, and the gray area above and below the dashed line represents the range of the uninfected control values. *P* values were calculated by Wilcoxon rank sum tests (for comparisons between groups) and Wilcoxon signed rank tests (for comparisons within groups), without adjustment for multiple comparisons. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, and $^{****}P < .0001$, for comparisons between the HIV groups, and $^{\#}P < .05$, $^{\#\#}P < .01$, and $^{\#\#\#}P < .0001$, for comparisons between HIV-infected and uninfected groups.

($P = .008$ and $P = .014$, respectively; Figure 1B). After cART, CSF sCD163 levels in participants in Fiebig stage III (6.5 ng/mL at 24 weeks) and chronic HIV infection (7.9 ng/mL at 48 weeks) decreased from pre-cART levels ($P < .001$ and $P = .003$, respectively) and were comparable to levels seen in uninfected controls (Figure 1B). Analysis of the longitudinal data from the

cohorts corroborated the aforementioned results. There were no changes in CSF sCD163 levels among participants in Fiebig stage I/II before and after cART, but there were declines in CSF sCD163 levels in participants in Fiebig stage III and chronic HIV infection after cART ($P = .001$ and $P = .003$, respectively).

Associations of Circulating sCD163 Levels With CNS Parameters Before cART

Our primary CNS outcome measures consisted of neuropsychological test performance scores (5 outcomes per Fiebig stage in the acute HIV-infected group), and our secondary outcome consisted of the following brain metabolites (5 outcomes in 4 brain regions, with 20 outcomes per Fiebig stage in the acute HIV-infected group): N-acetylaspartate and N-acetylaspartylglutamic acid, markers of neuronal health; myoinositol, a marker of astrocytosis and gliosis; choline, a marker cellular infiltration and inflammation/glial activation; glutamate and glutamine, markers of neuronal cell function; and creatine, a marker of energy metabolism. In Fiebig stage I/II before cART initiation, sCD163 levels correlated with positive CNS outcomes. Specifically, higher plasma sCD163 levels correlated with higher Grooved Pegboard *z* scores, an indicator of increased psychomotor performance (rho = 0.627; $P = .044$), and with higher glutamate and glutamine levels in frontal white matter (rho = 0.709; $P = .003$; Table 2 and Supplementary Figure 2). In Fiebig stage III before cART, sCD163 levels correlated with negative CNS outcomes, specifically higher plasma sCD163 levels correlating with lower N-acetylaspartate and N-acetylaspartylglutamic acid levels in the posterior cingulate gyrus (rho = −0.468; $P = .017$; Table 2 and Supplementary Figure 2). Multiple linear regression analyses were performed to explore the associations between CNS indices, sCD163 levels, and Fiebig stages. Evidence that associations with sCD163 levels differed between Fiebig stage I/II and III are shown in Table 2 and Supplementary Table 1. The positive association between plasma sCD163 levels and Grooved Pegboard scores before cART in Fiebig stage I/II differed from the association in Fiebig stage III (interaction $P = .017$), and similarly the positive association between plasma sCD163 levels and glutamate and glutamine levels in frontal white matter before cART in Fiebig stage I/II differed from the association in Fiebig stage III (interaction $P = .017$).

Similar trends were observed with the univariate correlations with regard to sCD163 levels in CSF. In Fiebig stage I/II, before cART, higher CSF sCD163 levels correlated with the positive CNS outcome of lower myoinositol levels in the basal

Table 2. Correlations Between Plasma and Cerebrospinal Fluid (CSF) Soluble CD163 (sCD163) Levels, in acute HIV infection, With Central Nervous System (CNS) Indices

Source, Time, acute HIV Fiebig Stage	CNS Index	Rho[a]	*P*[a]	CNS Outcome	*P*[b]
Plasma					
Before cART					
I/II	Grooved Pegboard *z* score Cognitive performance	0.627	.044	+	.017
I/II	Glutamate and glutamine in frontal white matter Neuronal cell function	0.709	.003	+	.017
III	N-acetylaspartate and N-acetylaspartylglutamic acid in posterior cingulate gyrus Neuronal health	−0.468	.017	–	.547
After cART					
III	Plasma neopterin Monocyte activation	0.399	.030	–	.479
CSF					
Before cART					
I/II	Myoinositol in basal ganglia Astrocytosis and gliosis	−0.569	.037	+	.156
I/II	Choline in basal ganglia Cellular infiltration	0.472	.043	–	.229
III	CSF neopterin Monocyte activation	0.479	.025	–	.913
After cART					
III	Neuropsychological *z*-4 score Cognitive performance	−0.522	.020	–	.053
III	N-acetylaspartate plus N-acetylaspartylglutamic acid in basal ganglia Neuronal health	−0.500	.031	–	.822

Abbreviations: cART, combination antiretroviral therapy; –, negative; +, positive.

[a]By Spearman correlation analysis.

[b]To provide evidence that associations with sCD163 levels differ between Fiebig stages I/II and III, *P* values from multiple linear regression models examining the interaction between circulating sCD163 levels and Fiebig stages with CNS indices as outcome variables are shown here. For complete multiple linear regression model data, see Supplementary Table 1.

ganglia (rho = −0.569; P = .037; Table 2 and Supplementary Figure 2). In Fiebig stage III, before cART, higher CSF sCD163 levels correlated with negative CNS outcomes, namely higher choline levels in the basal ganglia (rho = 0.472; P = .043) and higher CSF neopterin levels (rho = 0.479; P = .025; Table 2 and Supplementary Figure 2). In chronic HIV infection, both plasma and CSF sCD163 levels predominantly correlated with negative CNS outcomes, with the exception of higher plasma sCD163 levels correlating with lower choline levels in the frontal white matter (rho = −0.346; P = .049; Table 3 and Supplementary Figure 3). Before cART, higher plasma sCD163 levels correlated with lower N-acetylaspartate and N-acetylaspartylglutamic acid levels in the posterior cingulate gyrus (rho = −0.396; P = .026) and with higher plasma neopterin levels (rho = 0.350; P = .047; Table 3 and Supplementary Figure 3). Before cART, higher CSF sCD163 levels correlated in the frontal white matter with higher myoinositol levels (rho = 0.379; P = .019) and higher choline levels (rho = 0.324; P = .047), along with lower N-acetylaspartate and N-acetylaspartylglutamic acid levels in the frontal gray matter (rho = −0.402; P = .011) and higher CSF neopterin levels (rho = 0.356; P = .028; Table 3 and Supplementary Figure 3).

Post-cART Associations of Circulating sCD163 Levels With CNS Measures

Interestingly, no correlations were observed after cART initiation in Fiebig stage I/II. In Fiebig stage III, 24 weeks after cART, sCD163 levels continued to correlate with negative CNS outcomes. Higher CSF sCD163 levels correlated with lower neuropsychological z-4 scores, an indicator of decreased cognitive performance (rho = −0.522; P = .020) and with lower N-acetylaspartate and N-acetylaspartylglutamic acid levels in the basal ganglia (rho = −0.500; P = .031; Table 2 and Supplementary Figure 2). Higher plasma sCD163 levels correlated with higher plasma neopterin levels (rho = 0.399; P = .030; Table 2 and Supplementary Figure 2). Linear regression analyses showed mild evidence that, after cART, the positive association between CSF sCD163 levels and neuropsychological z-4 scores in Fiebig stage I/II differed from the association in Fiebig stage III (interaction P = .053; Table 2 and Supplementary Table 1). Despite the small number of observations, 3 of 9 associations show some evidence that Fiebig stage I/II and III differ in their association with sCD163 levels (Supplementary Table 1). In chronic HIV infection, 48 weeks after cART, sCD163 levels continued to associate with negative CNS outcomes: higher plasma sCD163 levels correlated with higher plasma neopterin levels (rho = 0.410; P = .004; Table 3 and Supplementary Figure 3).

Dynamics of CD163-Expressing Monocytes

To examine monocytes as a source of sCD163, we assessed cell-surface expression on the monocyte subsets. In participants in Fiebig stage I/II, cell-surface CD163 expression on classical, intermediate, and nonclassical monocytes remained unchanged before and after cART, and with the exception of classical monocytes 48 weeks after cART, surface CD163 levels were higher than in uninfected controls (P < .05 for all comparisons; Figure 2A–C). No differences were noted in cell-surface CD163 expression on the monocyte subsets between Fiebig stage I/II and their pre- or post-cART counterpart in Fiebig stage III (Figure 2A–C).

In Fiebig stage III, cell-surface CD163 expression remained unchanged on classical monocytes (Figure 2A), expression on intermediate monocytes decreased 24 weeks after cART (P = .023), and increased expression on nonclassical monocytes was observed after cART (P = .023 for 24 and 48 weeks; Figure 2B and 2C). The surface CD163 level on intermediate monocytes was higher in participants in Fiebig stage III, compared with uninfected controls (P = .005 before cART

Table 3. Correlations Between Plasma and Cerebrospinal Fluid (CSF) Soluble CD163 (sCD163) Levels During Chronic Human Immunodeficiency Virus Infection With Central Nervous System (CNS) Indices

Source, Time, CNS Index	Rho[a]	P[a]	CNS Outcome
Plasma			
Before cART			
Choline in frontal white matter, cellular infiltration	−0.346	.049	+
Acetylaspartate + N-acetylaspartylglutamic acid in posterior cingulate gyrus, neuronal health	−0.396	.026	–
Plasma neopterin, monocyte activation	0.350	.047	–
After cART			
Plasma neopterin, monocyte activation	0.410	.004	–
CSF			
Before cART			
Myoinositol in frontal white matter, astrocytosis and gliosis	0.379	.019	–
Choline in frontal white matter, cellular infiltration	0.324	.047	–
N-acetylaspartate plus N-acetylaspartylglutamic acid in frontal gray matter, neuronal health	−0.402	.011	–
CSF neopterin, monocyte activation	0.356	.028	–

Abbreviations: cART, combination antiretroviral therapy; –, negative; +, positive.

[a]By Spearman correlation analysis.

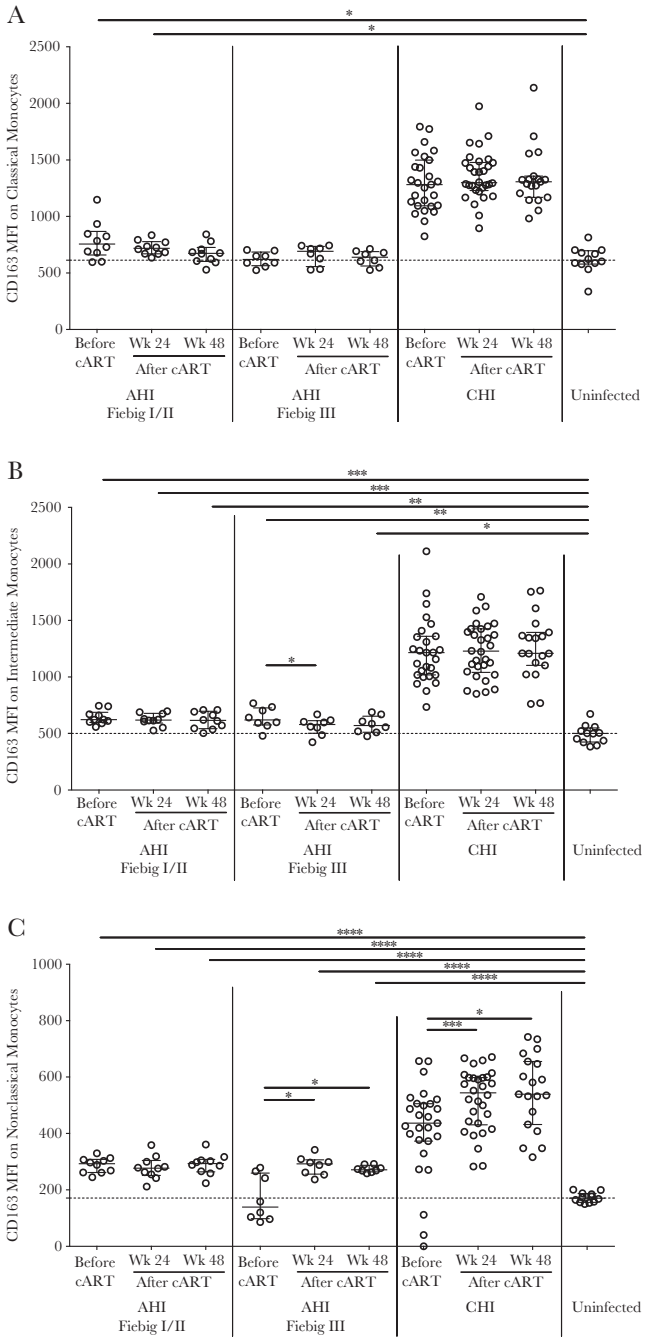

Figure 2. Geometric mean fluorescence intensity (MFI) of CD163 on classical (*A*), intermediate (*B*), and nonclassical (*C*) monocytes during acute human immunodeficiency (HIV) infection (AHI), by Fiebig stage; during chronic HIV infection (CHI), before and 24 and 48 weeks after combination antiretroviral therapy (cART) initiation; and in HIV- uninfected controls. Data are presented as medians and interquartile ranges. Dashed lines indicate the median level for HIV-uninfected controls. *P* values were calculated by Wilcoxon rank sum tests (for comparisons between groups) and Wilcoxon signed rank tests (for comparisons within groups), without adjustment for multiple comparisons. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001.

and *P* = .038 48 weeks after cART; Figure 2B). Surface CD163 levels on nonclassical monocytes were higher in participants in Fiebig stage III only after cART, compared with levels in uninfected controls (*P* < .0001 for 24 and 48 weeks; Figure 2C).

There were no differences in surface CD163 levels on classical monocytes between individuals in Fiebig stage III and uninfected controls (Figure 2A). In donors with acute HIV infection (ie, Fiebig stage I–III), cell-surface CD163 levels on all monocyte populations were lower than their chronically HIV-infected counterparts (*P* < .0001 for all comparisons; Figure 2A–C). Moreover, in participants with chronic HIV infection, cell-surface CD163 levels on all monocyte populations were higher than those in uninfected controls (*P* < .0001 for all comparisons; Figure 2A–C).

Association Between Monocyte Surface CD163 Expression and HIV-Related CNS Measures

Cell-surface CD163 levels on intermediate monocytes positively correlated with Grooved Pegboard *z* scores before cART in Fiebig stage I/II (rho = 0.928; *P* = .002) and with neuropsychological *z*-4 scores after cART in Fiebig stage III (rho = 0.738; *P* = .046; Table 4 and Supplementary Figure 4). There were no correlations between cell-surface CD163 expression on intermediate monocytes and MRS brain metabolite or sCD163 levels. Before cART in acute HIV infection (ie, Fiebig stage I–III), lower cell-surface CD163 expression on nonclassical monocytes correlated with higher plasma sCD163 levels (rho = −0.668; *P* = .008), and lower cell-surface CD163 expression on classical monocytes correlated with higher CSF sCD163 levels (rho = −0.664; *P* = .009; Supplementary Figure 5). Linear regression analyses demonstrated that the positive association between cell-surface CD163 expression on intermediate monocytes and Grooved Pegboard *z* scores in Fiebig stage I/II differed from the association in Fiebig stage III (interaction *P* = .001; Table 4 and Supplementary Table 2). When separating the acutely HIV-infected group into Fiebig stage I/II and Fiebig stage III, no correlations were noted. No correlations were observed between cell-surface CD163 expression on classical and nonclassical monocytes and CNS outcomes or sCD163 levels.

DISCUSSION

The RV254/SEARCH010 cohort allows for a unique opportunity to define the earliest CNS and systemic immunological changes that occur during acute HIV infection. In this study, we provide new evidence that cART initiation as early as Fiebig stage I/II may prevent the elevation of circulating sCD163 levels above those in HIV-uninfected individuals. Moreover, although cART administration during Fiebig stage III normalized sCD163 to levels observed in uninfected controls, the transient spike in sCD163 levels during acute infection prior to cART remained associated with detrimental CNS perturbations, with the primary and meaningful neurological outcome of poorer neuropsychological testing performance, a year later. Collectively these results highlight the importance of sCD163 as a crucial marker of neuronal injury that is relevant even as

Table 4. Correlations Between Cell-Surface CD163 Expression on Intermediate Monocytes, by Fiebig Human Immunodeficiency Virus Disease Stage, With Central Nervous System (CNS) Indices

Time	Fiebig Stage	CNS Index	Rho[a]	P^a	P^b
Before cART	I/II	Grooved Pegboard *z* score, cognitive performance	0.928	.002	.001
After cART	III	Neuropsychological *z*-4 score, cognitive performance	0.738	.046	.875

Abbreviations: cART, combination antiretroviral therapy.

[a]By Spearman correlation analysis.

[b]To provide evidence that associations with cell surface CD163 levels differ between Fiebig stages I/II and III, *P* values from multiple linear regression models examining the interaction between cell-surface CD163 levels on intermediate monocytes and Fiebig stages with CNS indices as outcome variables are shown here. For complete multiple linear regression model data, see Supplementary Table 1.

early as approximately 3 weeks after infection, with important implications for long-term neuronal health in this population.

Previous reports examining primary and chronic HIV infection have associated higher plasma sCD163 levels, not higher CSF sCD163 levels, with poor cognitive performance [10, 11, 31]. Here, we extend these findings in the setting of early acute HIV infection. Our data further highlight the association between myeloid activation and CNS perturbations and uncover a paradoxical relationship in Fiebig stage I/II before cART initiation. In this group, early myeloid activation correlated with positive CNS outcomes, suggesting that, during the earliest phase of the HIV insult, CD163 is being shed as a response to restorative tissue repair processes. CD163 shedding is a component of non-pathological biological processes, as evidenced by studies showing that intravenous injection of *Escherichia coli* endotoxin in healthy humans causes a rapid and transient increase in sCD163 and tumor necrosis factor α levels [8]. The restorative responses observed in Fiebig stage I/II contrast with observations in Fiebig stage III or chronic HIV infection, in which sCD163 may reflect an exacerbated or pathologic response. Although transient activation of myeloid cells is critical for wound healing responses, this activation may be hijacked in the context of HIV infection, leading to the immunological system becoming overwhelmed and the repair becoming unresolved.

Our previous report in chronically HIV-infected Thais found that $CCR2^+$ nonclassical monocytes were an independent index of cognitive impairment [19]. Our current data on cell-surface CD163 expression on nonclassical monocytes also implicate this subset in CNS perturbations during acute infection. Intermediate monocytes have been shown to preferentially cross an in vitro blood brain barrier model, suggesting that this subset may also be important in the pathogenesis of cognitive impairment [32]. Our data linking CD163 expression on intermediate monocytes and cognitive impairment suggest that this subset may be an important cellular source of sCD163. The fact that we noted associations between brain abnormalities and CD163 expression on all monocyte subsets and that there are alternate cellular sources of sCD163 (perivascular macrophages and microglial cells) highlights that the relationship between monocyte subsets, sCD163, and cognitive impairment is complex and warrants further study. Additionally, cART initiation differentially modulated surface CD163 expression on $CD16^+$ monocytes, adding to the complexity of the relationship. Higher cell-surface CD163 expression in chronic as compared to acute HIV infection may be due to immune-related influences. Serum interleukin 10 levels, which are increased during HIV disease progression [33], has the potential to increase CD163 expression on monocytes [34].

sCD163 has been associated with non–CNS-related complications, such as noncalcified coronary plaque [31], liver fibrosis and disease [35, 36], chronic kidney disease [37], and chronic lung disease [36]. Whether early cART initiation during Fiebig stage I/II can limit the development of these aforementioned HIV-associated comorbidities warrants continued investigation in this population. Literature on the biological relevance of sCD163 is very limited. One report has demonstrated that sCD163 has antimicrobial effects by recognizing staphylococci-bound fibronectin [38]. Other studies have shown that sCD163 can inhibit T-lymphocyte activation [39] and proliferation [40], highlighting an antiinflammatory or hyperactivation effect. Additionally, recent studies have revealed that plasma sCD163 can be found in 2 forms, a soluble ectodomain CD163 and an extracellular vesicle–associated CD163, which are components of separate myeloid cell responses in the context of systemic inflammation [37]. A deeper characterization of the composition and the biological role of sCD163 in HIV infection may offer potential prognostic value.

Our data argue for the initiation of cART early in Fiebig stage I/II to limit CD163 shedding that, in Fiebig stage III, appears to be associated with neuroinflammation and CNS injury. However, identifying HIV infection and starting treatment during the Fiebig I/II stages may prove challenging. For persons who are past the Fiebig stage I/II window, targeted strategies lowering myeloid activation may prove beneficial. Maraviroc, a C-C chemokine receptor type 5 (CCR5) antagonist, has been shown to decrease sCD163 levels while improving neuropsychological test results in chronically infected individuals with cART-suppressed viral loads [41]. While intensified therapy containing maraviroc (ie, the cART+ regimen) in our acute

HIV infection cohort did not significantly lower sCD163 levels as compared to participants receiving cART only (data not shown), antagonists that target multiple chemokine receptors, such as cenicriviroc (a dual CCR2 and CCR5 antagonist), may be beneficial. In a 48-week randomized study evaluating cenicriviroc versus efavirenz therapy in treatment-naive HIV-infected adults (clinical trials registration NCT01338883), levels of the monocyte activation marker sCD14 decreased with cenicriviroc and not efavirenz therapy [42].

Our study was in part exploratory, given our access to an unprecedented number of measures of inflammation and CNS measures and our desire to uncover unknown relationships to sCD163. A consequence, however, of our analysis is the generation of a large number of comparisons, potentially clouding data interpretation. With the generated *P* values, we do not wish to make inferences but rather highlight novel potential signals. We acknowledge that this is a limitation of the work and that further studies will be required to confirm the biological relevance of the discovered relationships. Other limitations of our study include the relatively small sample size and the significantly different ages and sex ratios across the cohorts. The lack of group matching for sex may not have had a significant impact on our study, as we did not note any significant differences when comparing sCD163 levels between sexes, and removing the female participants from the analysis did not appreciably alter the results (data not shown).

The characterization of biomarkers affords an opportunity for insight into acute HIV infection. Here, we show that early initiation of cART in Fiebig stage I/II appears to limit CD163 shedding, which may halt a neuroinflammatory cascade associated with brain abnormalities.

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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