Long-Term Suppressive Combined Antiretroviral Treatment Does Not Normalize the Serum Level of Soluble CD14

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Levels of soluble CD14 (sCD14) were longitudinally measured in 85 human immunodeficiency virus (HIV)–infected subjects during long-term receipt of suppressive combined antiretroviral therapy (cART) and compared to those in young and elderly HIV-negative control subjects. cART did not normalize sCD14 levels; rather, the HIV-infected group displayed a significantly higher sCD14 level at baseline (ie, before cART initiation), 1 year after cART initiation, and 5 years after cART initiation, compared with both control groups. Furthermore, the baseline CD4+ T-cell count was inversely associated with the baseline sCD14 level. Our results point to the necessity of complementary therapies to treat the activated/inflamed status associated with chronic HIV infection and to the benefits of early initiation of cART.

Keywords: soluble CD14; HIV infection; cART.

The introduction of combined antiretroviral therapy (cART) led to significant increases in the survival and quality of life of human immunodeficiency virus (HIV)–infected patients. However, the life expectancy of successfully treated HIV-infected subjects has been estimated to be 10 years lower than that of the general population [1]. It is known that cART is not able to normalize all immune-related parameters [2], even though full normalization of CD4+ T-cell counts can be achieved after persistent viral suppression [3]. In early 1998, an association between the level of soluble CD14 (sCD14) and HIV disease progression and associated clinical events was reported [4], and recently, sCD14 levels have been associated with mortality among HIV-infected subjects receiving cART [5]. We have reported that the sCD14 level but not the lipopolysaccharide (LPS) level was independently associated with markers related to progression of HIV disease, further supporting the clinical importance of sCD14 [6].

sCD14 is a marker for monocyte/macrophage activation and a mediator of bacterial LPS action [7]. Cross-sectional studies in different scenarios of HIV-treated infection have shown a higher sCD14 level in HIV-infected subjects, compared with healthy subjects [8, 9], although only a few longitudinal studies have explored the effect of a short-term cART on sCD14 level, with controversial results [10, 11]. To our knowledge, only 1 report analyzed the effect of long-term cART exposure on sCD14 level, but the immunovirological conditions of the study population at cART initiation were not considered [12]. The aim of this study was to analyze the effect of a long-term suppressive cART regimen on the sCD14 level.

SUBJECTS AND METHODS

Study Design

We analyzed serum samples from 85 consecutive cART-naive subjects with asymptomatic HIV infection who visited the Infectious Disease Unit of Virgen del Rocío University Hospital from April 1997 to April 2012. Available serum samples from HIV-infected subjects who initiated suppressive cART (ie, those with HIV-1 RNA levels of ≤40 copies/mL 3 months after the onset of cART) were analyzed at baseline (ie, before cART initiation; n = 85 samples) and after 1 year of suppressive cART (n = 85 samples). We also analyzed subjects with available samples after 5 years of suppressive cART (n = 43 samples). These samples were obtained from the HIV BioBank of the Spanish AIDS Research Network [13]. As reference values, we included samples from 18 HIV-negative age-matched donors who were ≤50 years old (young controls) and from 43 HIV-negative donors who were >50 years old (elderly controls). No control subjects had symptoms, none were receiving any treatment that could influence their immune status, and none had clinical data of active infections or
neoplasias. The study was approved by the ethics review board of the Virgen del Rocio University Hospital, and all study subjects provided written informed consent.

**Laboratory Measurements**

Absolute CD4+ T-cell numbers were determined in fresh blood, using an Epics XL-MCL flow cytometer (Beckman-Coulter, California, USA). Plasma HIV-1 RNA levels were measured by quantitative polymerase chain reaction (PCR; COBAS Ampliprep/COBAS Taqman HIV-1 test, Roche Molecular Systems, Basel, Switzerland) according to the manufacturer’s protocol. The detection limit for this assay was 40 copies/mL. Hepatitis C virus (HCV) RNA was detected by a commercially available PCR procedure (COBAS AmpliCegor, Roche Diagnosis [Barcelona, Spain]) with a detection limit of 15 IU/mL. sCD14 levels were quantified in duplicate from frozen serum samples that were thawed for the first time, using an enzyme-linked immunosorbent assay (R&D Systems, Abingdon, UK) according to the manufacturers’ protocol.

**Statistical Analysis**

All continuous variables were expressed as median values (interquartile range [IQR]). Categorical variables were expressed as the number (percentage) of cases. Differences between groups were analyzed using the χ² test, for categorical variables, and the Mann-Whitney U test, for continuous variables. Differences during the follow-up period were analyzed using the Wilcoxon test for paired variables. Correlations between quantitative parameters were analyzed using the Spearman ρ correlation coefficient test. The associations between the values of potential explanatory variables (eg, CD4+ T-cell count at baseline) and sCD14 level at baseline and the values at the 1-year and 5-year time points were assessed using a bivariate linear regression analysis. Variables determined to be statistically significant (P < .1) in the bivariate analysis were included in a multivariate analysis, using a step-forward procedure. The regression coefficient (B) and 95% confidence interval (CI) for B were estimated for this model. Statistical analysis was performed using SPSS, version 17 (SPSS [Chicago, IL]). A P value of < .05 was considered statistically significant.

**RESULTS**

**Characteristics of the Study Subjects**

Eighty-five HIV-infected subjects, 18 young controls, and 43 elderly controls were included in this study. All subjects were white. The baseline characteristics of HIV-infected subjects and control groups are shown in Table 1. The sCD14 level was analyzed at baseline for HIV-infected patients and controls and at 1 and 5 years for HIV-infected patients. During this period, the median increase in CD4+ T-cell count was 171 cells/mm³ (IQR, 86–240 cells/mm³) at 1 year and 353 cells/mm³ (IQR, 238–455 cells/mm³) at 5 years. For HIV-infected subjects, characteristics for the 85 subjects at baseline were not significantly different from those for the 43 subjects who remained enrolled at 5 years (data not shown).

**Long-Term cART Did Not Normalize the sCD14 Level in HIV-Infected Subjects**

When compared to young controls, HIV-infected subjects displayed a significantly higher sCD14 level at baseline, at 1 year, and at 5 years (P < .001 for all comparisons). Furthermore, the sCD14 level was neither reduced nor normalized at 1 year and 5 years, compared with the sCD14 level at baseline, because no significant changes were observed between time points (P = .152 and P = .381, by the Wilcoxon test, at 1 and 5 years, respectively; Figure 1A). Moreover, HIV-infected subjects displayed a significantly higher sCD14 level at all time points, compared with elderly controls (P < .001 for all comparisons). The elderly controls also displayed a higher sCD14 level than the young controls (P = .014; Figure 1A).

**Influence of Baseline Immunovirological Status on sCD14 Level**

We explored potential associations between sCD14 level and CD4+ T-cell count and HIV load at each time point of the analysis. A negative association between CD4+ T-cell count and sCD14 level was observed at baseline (r = −.425 [P = .031]; 5 years: r = 0.356 [P = .019]). We then further divided HIV-infected subjects into 2 groups according to their baseline CD4+ T-cell count: subjects in the low CD4+ T-cell count group had a baseline CD4+ T-cell count of ≤200 cells/mm³ (n = 26), and subjects in the high CD4+ T-cell count group had a baseline CD4+ T-cell count of >200 cells/mm³ (n = 59). Globally, when compared to the control groups, both subgroups of HIV-infected subjects had a higher sCD14 level at baseline, at 1 year, and at 5 years (P < .001 for all comparisons, except for the high CD4+ T-cell count group at 1 year vs the elderly controls [P = .094]; Figure 1D and 1E). Interestingly, only the low CD4+ T-cell count group had a reduction in sCD14 level at 5 years (P = .026, by the Wilcoxon test; P = .530 for the high CD4+ T-cell count group; Figure 1D and 1E). Interestingly, as we also found in a previous report [6], the baseline sCD14 level was significantly higher in the low CD4+ T-cell count group, compared with the high CD4+ T-cell count group (P < .001). In a similar analysis, we found that the baseline HIV load did not influence the changes in sCD14 level (data not shown).
Influence of cART Type on sCD14 Level
HIV-infected subjects were categorized into 3 subgroups according to the composition of their cART regimen (Table 1). Contrary to findings for the other groups, subjects receiving maraviroc-containing cART displayed similar baseline and 1-year sCD14 levels, compared with young controls (P > .05 for all comparisons; Supplementary Figure 1A). Notably, subjects receiving maraviroc-containing cART displayed the highest median CD4+ T-cell count (417 cells/mm³ [IQR, 356–362 cells/mm³]) compared with 289 cells/mm³ [IQR, 107–441 cells/mm³] for subjects receiving PI-sparing cART (P = .006) and 250 cells/mm³ [IQR, 107–452 cells/mm³] for those receiving PI-containing cART (P = .019)). Only subjects who received PI-sparing cART experienced a reduction in sCD14 level at 1 year (P = .032, by the Wilcoxon test).

Factors Associated With Baseline sCD14 Level and Changes in sCD14 Level During Follow-up
To identify the variables that were independently associated with changes in the sCD14 level during follow-up, we performed a multivariate analysis (Supplementary Table 1). Baseline sCD14 level was the only variable independently associated with changes in sCD14 level at 1 year (B, −0.621 [95% CI, −.813 to −.430]; P < .001) and 5 years (B, −0.911 [95% CI, −1.107 to −.715]; P < .001). We also analyzed potential variables that could be associated with baseline sCD14 level, and only baseline CD4+ T-cell count was independently associated (B, −0.001 [95% CI, −.001 to 0.000]; P < .001).

DISCUSSION
In this longitudinally designed study, we report that long-term receipt of suppressive cART (for up to 5 years) neither reduced nor normalized the level of sCD14, which is a soluble marker of inflammation and innate immune activation and has been associated with all-cause mortality in HIV infection [4–6]. First, it is important to note that our study population included subjects for whom the time since diagnosis was short, who initiated cART early, and who received virologically successful long-term regimens, suggesting that serious damage responsible for the elevated sCD14 level occurs very early during HIV infection and persists despite viral suppression and restoration of CD4+ T-cell count. Second, the sCD14 level remained even higher in treated patients than in HIV-negative elderly controls who were, on average, >30 years older. Hence, one could speculate that the sCD14 level could be another
immunosenescence-associated marker, like those present in treatment-naive HIV-infected subjects with a short period of infection [14].

Some longitudinal analyses considering the short-term effect of cART exposure found that the sCD14 level was not normalized [10, 11], although 2 years of treatment yielded a significant reduction [10]. To our knowledge, only 1 longitudinal analysis has previously reported the effects of long-term cART exposure, showing that the sCD14 level declined with exponential decay after a median follow-up time of 6.5 years, although the authors were unable to predict whether, beyond the study period, the sCD14 level would plateau [12]. Our results argue in favor of a plateau in sCD14 level after cART, compared with levels for both young and elderly controls.

Potential mechanisms that could explain a plateau in the sCD14 level during HIV infection include (1) persistent micr

Figure 1. Influence of immunovirological parameters on the level of soluble CD14 (sCD14). A, sCD14 levels in all human immunodeficiency virus (HIV)-infected subjects studied at baseline (ie, before combined antiretroviral therapy [cART] initiation), 1 year after cART initiation, and 5 years after cART initiation and in control groups of young and elderly individuals. Note that, when the 2 potential outliers in the young control group were omitted, differences between young and elderly control groups became less significant ($P = .055$). Interestingly, one of these outliers had the highest CD4$^+$ T-cell count, but the other value was not available. B and C, The relationships between sCD14 level and CD4$^+$ T-cell count (B) or HIV load (C) at baseline were evaluated by the Spearman $\rho$ correlation coefficient test. B, The sCD14 level in HIV-infected subjects with a low baseline CD4$^+$ T-cell count ($\leq 200$ cells/mm$^3$), at baseline, at 1 year, and 5 years, and in control groups. C, The sCD14 level in HIV-infected subjects with a high baseline CD4$^+$ T-cell count (>200 cells/mm$^3$), at baseline, 1-year cART, 5-years cART, and control groups. The Wilcoxon test (dashed line) was used to analyze differences during the follow-up period (ie, at 1 and 5 years after cART initiation). The Mann–Whitney $U$ test (solid line) was used to compare the level of sCD14 between HIV-infected subjects and healthy subjects. * $P < .05$ and ** $P < .001$. 

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therapy, the subjects maintained an increased sCD14 level [11]. Similarly, we found no changes in sCD14 level after a median duration of suppressive cART of 3.5 years in HIV-infected populations with a low baseline CD4+ T-cell count [15]. However, subjects who started cART with a high CD4+ T-cell count, such as those who received maraviroc-containing cART, showed sCD14 levels similar to those of the HIV-negative control group. This may explain why we did not observe the potential immunomodulatory effect of this CCR5 antagonist. However, we cannot exclude such effect in highly immunodeficient patients starting a maraviroc-containing regimen.

Our study has several limitations. First, there was a low number HIV-infected patients who were followed up at 1 year. This number was even lower at the 5-year time point, although the missing data corresponded to patients who had not reached the 5-year time point. Also, subjects eligible for enrollment as control subjects were scarce, complicating the achievement of statistical power. Moreover, we cannot exclude the possibility that the sCD14 level could normalize after a longer period of cART. Nevertheless, our results strongly recommend early initiation of cART to control immune damage beyond sCD14 levels, and they point to the necessity of searching for complementary therapies to treat the activated/inflamed status associated with chronic HIV infection.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank the patients participating in this study; the Infectious Diseases Unit, Virgen del Rocío University Hospital, the HIV BioBank, which is integrated in the Spanish AIDS Research Network; and Juan Manuel Praena Fernández, for statistical assistance.

Financial support. This work was supported by the Spanish AIDS Research Network of Excellence (grants RD12/0017/0029 and RD12/0017/0037), the Ministerio de Sanidad, Política Social e Igualdad (grant EC11-520), Fundación Progreso y Salud (grant PI-0081-2011), and the Fondos de Investigación Sanitaria (grant P11/02014 and Miguel Servet grant CP07/00240 to Y. M. P., grants P11/02014 and P112/02283, grant CP08/0172 to E. R.-M., and Sara Borrell grant CD10/00382 to S. F.-M, and Y.M.P. also received a Nicolas monard Grant from Conselleria de Salud y Bienestar Social de Junta de Andalucía (C-0010/13), and Pfizer/ViiV Healthcare (grant number W5843473).

Potential conflicts of interest. M. L. has received a grant from Pfizer. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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