High Soluble CD14 Levels at Primary HIV-1 Infection Predict More Rapid Disease Progression

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The soluble CD14 (sCD14) level was found associated with mortality during the chronic phase of human immunodeficiency virus (HIV) infection. Here we assessed its prognostic value in 138 patients with primary HIV infection. Higher sCD14 levels were associated with death, from myocardial infarction, but this was based on 3 deaths only. Among 68 untreated patients, those with higher sCD14 levels had more rapid spontaneous CD4 cell decline during the first 18 months following primary infection. This association persisted after adjustment for age, the CD4 cell count, and HIV viral load at diagnosis.

Keywords. HIV-1; immune activation; mortality; sCD14; systemic inflammation.

A variety of biomarkers (inflammatory and anti-inflammatory cytokines, chemokines, monocyte activation markers, etc.) have been implicated in the inflammation/immune activation associated with human immunodeficiency virus (HIV) infection. Biomarkers of cell activation and coagulation, such as high-sensitivity C-protein, interleukin (IL)–6, and D-dimer are predictive of opportunistic disease onset [1] and both AIDS-related and non-AIDS mortality [2]. Systemic inflammation and monocyte/macrophage activation within the arterial wall of HIV-infected patients are both associated with cardiovascular disease and arterial inflammation [3]. High levels of tumor necrosis factor (TNF)–α, IL-10, and monocyte chemoattractant protein (CCL2, also known as MCP-1) are also associated with HIV disease progression [4]. Elevated interferon γ–induced protein 10 (CXCL10, also called IP10) levels were recently linked to immune activation and the short-term CD4 cell count decline in untreated patients followed since primary infection [5].

The earliest and principal site of inflammation/immune activation during HIV infection is the gastrointestinal tract, which houses the largest immune system in the body. Microbial translocation is defined by systemic release of lipopolysaccharide (LPS), a major component of Gram-negative bacteria with immunostimulatory properties. LPS stimulation leads CD14+ monocyte/macrophages to secrete soluble CD14 (sCD14), which binds to LPS [6]. There is some evidence that sCD14 might also be produced during primary infection, earlier than other biomarkers of microbial translocation such as intestinal fatty acid binding protein and peptidoglycan [7].

During the chronic phase of HIV infection, higher plasma sCD14 levels are associated with immune activation, intestinal dysbiosis, and microbial translocation [8]. Elevated sCD14 levels are also predictive of all-cause mortality in patients with chronic HIV infection [9], but there are no data on the impact of sCD14 on the risk of disease progression during primary infection, in terms of either mortality or the rate of CD4 cell decline.

The aim of this study was to measure markers of inflammation and immune activation during primary HIV infection and to determine the impact of sCD14 on mortality and long-term CD4 cell count kinetics during follow-up in the ANRS PRIMO cohort.

METHODS

Study Population
The multicenter ANRS PRIMO cohort [10] was approved by the Paris-Cochin Ethics Committee, and all patients gave their written informed consent. Primary HIV infection was confirmed by detection of p24 antigenemia or plasma virus plus a negative or weakly reactive enzyme-linked immunosorbent assay (ELISA), or an evocative incomplete Western blot, or an interval of less than 3 months (6 months before 2002) between a negative and positive ELISA. All the patients were antiretroviral-naive at enrollment, and no specific recommendations on treatment initiation were provided.
Here we analyzed data for 138 patients who received combination antiretroviral therapy (cART) either immediately on primary-infection diagnosis (n = 70) or at least 1 year after primary-infection diagnosis (n = 68), treated efficiently (viral load <50 copies/mL) for at least 3 years, and who had available serial frozen samples.

**Cytokine and Chemokine Measurements**

We quantified 9 soluble proteins with inflammatory properties (IL-1α, IL-6, CXCL10, and TNF-α), anti-inflammatory properties (latency-associated peptide [LAP] and IL-10), or a relationship with monocyte activation (CCL2, sCD14, and soluble CD163–sCD163). Levels of IL-1α, CXCL10, TNF-α, LAP, IL-10, and CCL2 were measured with the FlowCytomix bead-based multiplex immunoassay (eBioscience Inc, San Diego, California), according to the manufacturer’s instructions. Levels of IL-6, sCD14, and sCD163 were measured with specific ELISA assays (Human IL6 Platinum ELISA, eBioscience; Human CD14 DuoSet ELISA and Human CD163 DuoSet ELISA, R&D Systems, Minneapolis, Minnesota). Each determination was performed in duplicate. Samples with undetectable levels of a given analyte were arbitrarily attributed half the minimal detectable value.

**Statistical Analysis**

Baseline characteristics were compared using the χ² test and the Wilcoxon rank-sum test for categorical and continuous variables, respectively. When necessary, continuous variables were categorized around the median of observed values, or using published cut-off values. Spearman’s nonparametric correlation coefficient was used to estimate associations between 2 continuous variables. Survival after the estimated date of infection was assessed with the Kaplan–Meier method according to baseline biomarker levels dichotomized around the median. Survival curves were compared with the log-rank test. The spontaneous decline in the CD4 cell count was assessed in the 68 untreated patients. The CD4 cell decline (on a square-root scale) was an-
found to be associated with the rate of CD4 cell decline (Figure 2): patients with baseline sCD14 >1258 ng/mL had a significantly steeper CD4 slope during the first 18 months after primary infection than those with sCD14 ≤1258 ng/mL ($P = .001$). For example, in a patient with sCD14 >1258 ng/mL and a patient with sCD14 ≤1258 ng/mL, both with a baseline CD4 cell count of 500/µL, the respective average CD4 cell loss after 18 months of follow-up without cART would be 169/µL.
The rate of CD4 cell decline was similar in the 2 sCD14 groups beyond 18 months, leading to a sustained difference in the CD4 cell count. Similar results were obtained after adjusting for age and the baseline HIV-RNA level and CD4 cell count. No statistically significant difference in the spontaneous CD4 cell decline was observed according to the levels of the other markers (ie, CXCL10, CCL2, TNF-α, IL-1α, IL-6, sCD163, IL-10, and LAP), and according to the HIV viral load (≥5 log vs <5 log_{10} copies/mL; P = .3).

**DISCUSSION**

We explored whether levels of inflammatory cytokines, anti-inflammatory cytokines and chemokines, and monocyte activation markers at diagnosis of primary HIV infection influenced subsequent disease progression. Only higher sCD14 levels were associated with a higher risk of death in both treated and untreated patients, and, in untreated patients, with a steeper CD4 cell decline during the first 18 months, leading to a sustained difference in the CD4 cell count. This relation between the rate of CD4 cell decline and the baseline sCD14 level persisted after adjustment for the baseline CD4 cell count and viral load.

The association between sCD14 and mortality found in our study should be interpreted with caution, as it was based on 3 deaths only. Monocyte activation and inflammation are known to increase with age; given the age at enrollment of 2 of the 3 patients who died, age could also be playing a role in their deaths.

In the SMART study, higher sCD14 levels were associated with all-cause mortality in patients with chronic HIV infection. Translocation of microbial products directly contributes to systemic immune activation and CD4 cell depletion, and may ultimately influence the rate of HIV disease progression [11]. However, microbial translocation might not be the main cause of heightened sCD14, especially in primary HIV infection as reported by Chevalier et al [7]; they also reported that IL-1RA and sCD14 levels at the time of primary HIV infection predict the T-cell activation set-point. High sCD14 levels could lead to increased CD38 expression on CD4 and CD8 T cells, which has been implicated in CD4 cell loss and mortality [11, 12].

Conflicting results have been reported in the literature; CXCL10 (also known as IP-10) was found to be associated with the CD4 decline below 350/µL in the 6 months following primary infection [5], while in another study this association was not confirmed [13]. These results were based on an approach where CD4 counts were categorized as a binary outcome. Mixed-effect models used in our study take into account the correlation between measurements in a given subject and more importantly use the whole information (all the CD4 measurements), providing a greater power than when the outcome
is dichotomized according to a CD4 threshold such as 350 CD4 cells/µL.

sCD14 was recently proposed as a surrogate marker for the extent of subclinical vascular disease associated with coronary artery calcification in cART-treated patients with chronic HIV infection [14]. This might have implications for the 3 deaths, all from myocardial infarction, that occurred among our patients with high sCD14 levels. In another study, increased arterial inflammation, detected by fluorodeoxyglucose-positron emission imaging, was associated with elevated circulating markers of macrophage activation, but sCD14 levels were not measured [3]. Importantly, high sCD14 levels are associated with an increased risk of cardiovascular events and mortality in clinical situations other than HIV infection [15].

In conclusion, this is the first study to explore whether sCD14 levels measured during primary HIV infection influence subsequent disease progression. We found that baseline sCD14 levels were associated with more rapid CD4 cell decline and with a higher risk of death from coronary heart disease. If confirmed in larger studies, these findings should help to identify patients at risk of disease progression.

Notes

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