Short communication

Circulating interleukin-6 levels correlate with residual HIV viraemia and markers of immune dysfunction in treatment-controlled HIV-infected patients

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Background: Antiretroviral therapy (ART)-controlled HIV-infected patients have elevated levels of systemic inflammatory markers, C-reactive protein (CRP) and interleukin (IL)-6, which correlate with increased cardiovascular risk and/or mortality. Persistent low-level viral replication could be involved in this inflammatory state. We evaluated whether residual viral load (VL) correlated with the level of systemic inflammatory/immune markers in ART-controlled HIV-infected patients.

Methods: We evaluated 122 antiretroviral-controlled patients with VL 1–500 copies/ml for circulating levels of high-sensitivity (hs)CRP, hsIL-6, IL-8, soluble (s)CD14 and soluble tumour necrosis factor (TNF) receptors, sTNFR1 and sTNFR2.

Results: The patients were 80.3% men, the median age was 47 years, the median CD4+ T-cell count was 519 cells/mm³, the median nadir CD4+ T-cell count was 180 cells/mm³, the median VL was 28 copies/ml and the median body mass index was 23.3 kg/m². The median (range) values for IL-6, CRP, IL-8, sCD14, sTNFR1 and sTNFR2 were 0.685 pg/ml (0.15–5.46), 1.8 mg/l (0.2–9.7), 10.0 pg/ml (1.6–71.1), 1,174 ng/ml (214–3,145), 1,112 pg/ml (583–5,834) and 2,412 pg/ml (1,142–7,688), respectively. IL-6 values correlated positively with HIV VL (rho=0.217, P=0.017). The VL threshold value for significantly increased IL-6 was 31 copies/ml (P=0.023). IL-6 values correlated with markers of immune dysfunction: the CD4/CD8 ratio (rho=-0.248, P=0.011), CD4 nadir level (rho=-0.186, P=0.04) and nadir CD4/CD8 ratio (rho=-0.257, P=0.008). They negatively correlated with markers of immune activation sCD14 (rho=-0.236, P=0.011) and IL-8 (rho=-0.290, P=0.002). We found no correlation between VL and CRP or other markers of inflammation/immune dysfunction including sTNFR1, sTNFR2, sCD14 and IL-8.

Conclusions: We report here that low-range IL-6 levels correlated with low-range VL and inversely with sCD14 and IL-8. Our findings suggest that maintaining VL<30 copies/ml in HIV-infected patients might therefore reduce IL-6.

Introduction

HIV-infected patients have elevated levels of systemic inflammatory markers [1]. Upon treatment with antiretroviral therapy (ART), the levels of tumour necrosis factor (TNF)-α and soluble (s)TNF receptors decrease; however, several studies report that C-reactive protein (CRP) and interleukin (IL)-6 levels do not change [2–4]. Even in long-term ART-treated patients, it has been reported that IL-6 and CRP levels remain higher than in age-matched non-infected subjects [5]. Crucially, these elevated IL-6 and/or CRP levels in ART-controlled patients correlate with increased cardiovascular risk and/or mortality in most studies [1,6–9]. We would therefore like to understand better the causes of elevated IL-6 and CRP in these patients. One hypothesis is that persistent viral replication or release of virus from infected cells causes release of these factors resulting in low-grade systemic inflammation. Indeed, a relationship between viral load (VL) and...
IL-6, but not CRP, was previously reported in viraemic patients (VL > 400 copies/ml) [6,10]. This relationship has not yet been evaluated at low VLs. In this study, we evaluated, in ART-controlled HIV-infected patients with a VL of < 500 copies/ml, whether residual VL correlated with the level of systemic inflammatory and immune markers.

Methods

Patients

We enrolled 132 successive ART-treated HIV-infected patients with CD4+ T-cell counts > 200 cells/mm³ and a VL of 1–500 copies/ml from outpatients of the Departments of Infectious Diseases and Internal Medicine at Pitié-Salpêtrière Hospital (Paris, France). Of these patients, 10 were excluded due to CRP levels > 10 mg/ml (suggestive of other inflammatory processes according to the American Heart Association [11]).

VL was routinely evaluated by using the Cobas AmpliPrep/CobasTaqman HIV-1 test version 2 (Roche Diagnostics, Meylan, France), which has a limit of quantification of 20 copies/ml. In patients with VL < 20 copies/ml, residual plasma viraemia was measured by using an ultra-sensitive assay able to detect 1 copy of HIV RNA/ml [12] on a frozen aliquot of the same plasma. The median VL was 28 copies/ml (range 1–497).

Markers of inflammation

High-sensitivity (hs)CRP was measured by immunonephelometry (IMMAGE; Beckman-Coulter, Villepinte, France). hsIL-6, IL-8, sCD14, and sTNF-α receptors R1 and R2 (sTNFR1 and sTNFR2) levels were determined by ELISA (R&D Systems, Lille, France).

Statistical tests

Mann–Whitney U, Spearman and ANOVA tests were used as appropriate. Values of \( P < 0.05 \) were considered statistically significant. We determined the cutoff value of VL for which the IL-6 levels were significantly different.

Results

Of the 122 patients, 80.3% (\( n = 98 \)) were men, the median age was 47 years (range 21–80), the median CD4+ T-cell count was 519 cells/mm³ (range 212–1,389), the median nadir CD4+ T-cell count was 180 cells/mm³ (range 1–891), the median CD8+ T-cell count was 842 cells/mm³ (range 33–2,810), the median CD4/CD8 ratio was 0.615 (range 0.08–23.91) and the known median duration of infection was 162 months (range 1–351). A total of 30 (25%) patients were stage C. The median body mass index was 23.3 kg/m² (range 16.9–34.0). The HCV status was recorded in 118 patients, 101 were HCV-negative and 17 HCV-positive. The median duration of ART was 126 months (range 1–250). The ART consisted of nucleoside reverse transcriptase inhibitors (NRTIs) for 112 (92%) patients, non-NRTIs for 32 (26%), protease inhibitors for 88 (72%), raltegravir for 15 (12%), maraviroc for 7 (6%) and T20 for 1.

The median and mean IL-6 values of the 122 patients tested were 0.685 and 1.04 pg/ml (sd 1.01, range 0.15–5.46). Due to the lack of normal values, we evaluated IL-6 levels in 15 healthy controls. The median and mean IL-6 values from controls were in the same range: 1.01 and 1.14 pg/ml (sd 0.67, range 0.1–2.29). The median CRP value was 1.8 mg/l (0.2–9.7), which is in the normal range; median IL-8 levels were 10.0 pg/ml (1.6–71.1), sCD14 levels were 1,174 ng/ml (214–3,145), sTNFR1 levels were 1,112 pg/ml (583–5,834) and sTNFR2 levels were 2,412 pg/ml (1,142–7,688). The distribution of VL, IL-6, CRP, sTNFR1 and sTNFR2 was not normal; therefore, the correlations were evaluated by using non-parametric tests. Similar results were obtained when the values were log-transformed (data not shown).

VL was not related to duration of ART or time to diagnosis.

IL-6 values correlated positively with HIV RNA levels (rho=0.217, \( P = 0.017 \); Figure 1). The VL threshold value for significantly increased IL-6 was 31 copies/ml (\( P = 0.023 \)). IL-6 correlated positively with no other marker of inflammation or immune activation and even correlated negatively with sCD14 and IL-8 (Table 1). Importantly, IL-6 values, but not other markers, correlated with markers of immune dysfunction: the CD4/CD8 ratio (rho=–0.248, \( P = 0.011 \)), the CD4+
Circulating IL-6 levels correlate with residual HIV viral load

T-cell nadir level (rho=-0.186, \( P = 0.04 \)) and the nadir CD4/CD8 ratio (rho=-0.257, \( P = 0.008 \)). By contrast, levels of CRP did not correlate with VL or with any other inflammatory or immune activation marker. This was the case when considering the median CRP value (1.8 mg/l) or when considering values reflecting a low (<1 mg/l), average (1–3 mg/l) or high (>3 mg/l) cardiovascular risk, according to the American Heart Association [11] (Figure 2).

The levels of sTNFR1 and sTNFR2, sCD14 and IL-8 did not correlate with VL or with the immune indicators CD4, CD8, ratio CD4/CD8, nadir CD4+ T-cell count and nadir CD4/CD8 (except sTNFR1 and current CD4; rho=0.203, \( P = 0.026 \)). The levels of IL-8, a chemokine associated with innate immune activation and inflammation and sCD14, a marker of immune activation, correlated strongly. Both sTNFR1 and sTNFR2 also correlated strongly with sCD14 and IL-8 (Table 1). sTNFR2 but no other marker was higher in HCV-positive patients (2,935 versus 2,346 pg/ml in HCV-positive versus HCV-negative patients; \( P = 0.006 \)).

Discussion

We report here, for the first time, that VL in the low range correlated with low range IL-6 levels. We found no correlation between VL and CRP or other markers of inflammation/immune dysfunction including sTNFR1 and sTNFR2, sCD14 and IL-8. In this group of controlled patients, the median IL-6 and CRP levels were in the normal range.

The Strategies for Management of Anti-Retroviral Therapy (SMART) study found a direct correlation between the level of IL-6 and loads of HIV viraemia above 400 copies/ml, but the level of IL-6 was found to be similar whether the VL was less than or greater than 400 copies/ml [5,6,10], suggesting that VL drives IL-6 production at high loads; however, in previous studies the researchers did not look at a range of loads below 400 copies/ml because the available tests were not sufficiently sensitive. To evaluate low-level viraemia, we used an ultra-sensitive assay, which is capable of detecting 1 copy/ml [12]. We are thus able to report here that the VL of HIV correlates with levels of IL-6 also at very small loads of virus. The threshold at which IL-6 levels are significantly increased is at a VL of 31 copies/ml. Because elevated IL-6 levels are thought to be a risk factor for cardiovascular disease (CVD) and mortality, both in the general population and in HIV-infected patients [6], this suggests that maintaining a VL below 30 copies/ml could be beneficial with regard to inflammation.

IL-6 levels possibly reflect ongoing inflammation in the arterial wall resulting in instability of existing

### Table 1. Correlation between HIV VL and inflammatory and immune activation markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>CRP</th>
<th>sTNFR1</th>
<th>sTNFR2</th>
<th>IL-8</th>
<th>sCD14</th>
<th>VL</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>0.072 (( P = 0.426 ))</td>
<td>0.155 (( P = 0.090 ))</td>
<td>-0.023 (( P = 0.801 ))</td>
<td>-0.290 (( P = 0.002 ))</td>
<td>-0.236 (( P = 0.011 ))</td>
<td>0.217 (( P = 0.017 ))</td>
</tr>
<tr>
<td>CRP</td>
<td>–</td>
<td>0.074 (( P = 0.418 ))</td>
<td>0.079 (( P = 0.388 ))</td>
<td>0.005 (( P = 0.961 ))</td>
<td>0.075 (( P = 0.417 ))</td>
<td>-0.007 (( P = 0.939 ))</td>
</tr>
<tr>
<td>sTNFR1</td>
<td>–</td>
<td>–</td>
<td>0.724 (( P = 0.0001 ))</td>
<td>0.169 (( P = 0.069 ))</td>
<td>0.289 (( P = 0.002 ))</td>
<td>-0.057 (( P = 0.534 ))</td>
</tr>
<tr>
<td>sTNFR2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.324 (( P = 0.001 ))</td>
<td>0.403 (( P = 0.001 ))</td>
<td>0.093 (( P = 0.309 ))</td>
</tr>
<tr>
<td>IL-8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.562 (( P = 0.0001 ))</td>
<td>-0.127 (( P = 0.171 ))</td>
</tr>
<tr>
<td>sCD14</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>-0.015 (( P = 0.870 ))</td>
</tr>
</tbody>
</table>

**Note:** CRP, C-reactive protein; IL, interleukin; sCD14, soluble CD14; sTNFR1, soluble tumour necrosis factor (TNF) receptor 1; sTNFR2, soluble tumour necrosis factor (TNF) receptor 2; VL, viral load.
plagues and thereby increasing the risk of clinical manifestation of CVD. IL-6 may be elevated due to many factors. For example, HIV replication or specific HIV proteins such as Tat can activate inflammatory pathways in infected cells leading to IL-6 production [13]. We show here that low levels of VL correlate specifically with the systemic level of IL-6. We find that IL-6 levels are also related to immune markers indicative of the severity of HIV infection and/or insufficient immune reconstitution (nadir CD4+ T-cell count, CD4/CD8 ratio and nadir CD4/CD8 ratio). A low nadir CD4+ T-cell count and CD4/CD8 ratio <1, as well as HIV RNA levels, have been linked previously to an increased occurrence of myocardial infarction in ART-treated HIV-infected patients [14]. This makes plausible the hypothesis that IL-6 might provide a causal link between immune dysfunction and non-AIDS-related morbidity/mortality in HIV-infected patients.

Circulating levels of CRP do not correlate with HIV RNA either at VL>50 copies/ml [5,10,15] or, as observed here, at low VL. Nevertheless, a role for CRP in increased mortality and risk of CVD has been found in several studies of HIV-infected patients [6–9]. This suggests that factors indirectly related to the viral infection are responsible for increased CRP levels in well-controlled HIV-infected patients.

We found that the levels of sCD14 and IL-8 – markers of immune activation – did not correlate with low level VL, IL-6 or CRP but correlated strongly with sTNFR1 and R2 indicating a link between activation of innate immunity and of the TNF-α system. IL-6 and CRP must, therefore, be regulated in well-controlled HIV-infected patients by mechanisms other than activation of the TNF-α system. Moreover, we found that the levels of IL-6 were negatively associated with markers of immune activation. In the SMART study, sCD14 levels correlated with those of IL-6 and CRP and were linked to increased mortality [10]. We have no explanation for this discrepancy. Therefore, decreasing IL-6, which could be beneficial for the cardiovascular risk, might be counterbalanced by increased sCD14 with possible deleterious consequences [10].

Our results have several limitations. The association between IL-6 and VL, although significant, has a low rho value and therefore its clinical relevance could be discussed. We have no evidence for causal relationships. Our data need to be confirmed with an independent group of HIV-infected patients and compared with a paired-group of non-infected subjects.

In conclusion, our findings suggest that maintaining undetectable VL in HIV-infected patients might reduce IL-6 levels. Further studies are required to document a beneficial effect to avoid long-term inflammation-related complications.

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Disclosure statement

The authors declare no competing interests.

References


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