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IL-2 cycling causes transient increases in hsCRP and D-dimer that are not associated with plasma HIV-RNA levels

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Abstract

Objective—To determine the effects of interleukin (IL)-2 treatment on inflammatory and thrombotic biomarkers in chronically HIV-infected adults receiving antiretroviral therapy (ART).

Methods—Cryopreserved plasma was evaluated retrospectively for CRP and D-dimer at baseline, end of an IL-2 cycle, and long-term follow-up from 2 randomized, controlled trials: 1) 57 IL-2-naïve adults receiving either 3–6 cycles of IL-2 plus ART (nucleoside analogues) or ART alone for 12 months; 2) 40 IL-2-experienced adults on HAART who either interrupted or continued therapy for

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Conflicts of Interest: The US Government has been granted a patent for the use of intermittent subcutaneous IL-2 as therapy in HIV infection, listing H. C. Lane and J.A. Kovacs as co-inventors.

Prior Publication Disclosure

This work was presented in an abstract format at the 16th Conference on Retroviruses and Opportunistic Infections (CROI), held in Montreal, Canada from 2/8/09 to 2/11/09.

6 months after a baseline IL-2 cycle. High-sensitivity CRP (hsCRP) was measured by immunonephelometry (detection limit 0.175 mg/L) and D-dimer by latex agglutination (detection limit 0.20 mg/L). Median within-group differences and pre- and post-IL-2 changes between groups were assessed via non-parametric Wilcoxon Signed-Rank and Mann-Whitney U tests. Spearman Rank test was used to assess correlations between changes in hsCRP, D-dimer, and HIV-RNA viral load.

Results—Significant increases in hsCRP (Study 1: 138.6 mg/L; Study 2: 58.9 mg/L) and D-dimer (Study 1: 3.1 mg/L; Study 2: 0.4 mg/L, all $p < 0.0001$) occurred by the end of the initial IL-2 cycle, returning to baseline by end of study. No correlations were seen between changes in hsCRP or D-dimer and HIV-RNA, CD4 T cell count or proliferation (Ki67 expression). No thrombotic or cardiovascular serious adverse events occurred during these study periods.

Conclusions—IL-2 dosing caused transient increases in plasma hsCRP and D-dimer levels, unassociated with HIV-RNA viral load, suggesting the possibility of increased risk for thrombotic events.

Keywords

Interleukin-2; C-reactive protein; D-dimer; HIV infections; Randomized Controlled Trial

Introduction

Several Phase I/II studies have demonstrated that intermittent cycling of IL-2 leads to long-term CD4 T cell increases in chronic HIV-infection [1,2]. Recently, data from large-scale multicenter phase III trials (ESPRIT and SILCAAT) [3,4] showed that these CD4 T cell increases are not associated with clinical benefit and may be associated with more Grade 4 clinical events [5,6].

IL-2 causes the release of pro-inflammatory cytokines, including tumor necrosis factor (TNF)- α , IL-1 β , and IL-6 [7–10], which are known to increase acute phase reactants such as C-reactive protein (CRP) and activate pro-thrombotic pathways [11,12]. Multiple studies have established associations between levels of CRP and the fibrinogen breakdown product D-dimer with both cardiovascular and thrombotic disease [10,13–15]. These biomarkers have also been shown to be elevated in HIV-infected patients [16,17]. Recent findings from the Strategies for the Management of Antiretroviral Therapy (SMART) trial demonstrated that baseline levels of CRP and D-dimer were predictors of all-cause mortality in a chronically HIV-infected sample of patients who were undergoing highly active antiretroviral treatment (HAART) interruption [18]. Understanding the acute and chronic effects of IL-2 on these markers may thus provide insights into potential IL-2-related complications.

In this study, plasma CRP and D-dimer levels were analyzed longitudinally in 2 randomized clinical trials that evaluated IL-2 as an adjuvant therapy in HIV-infection. The first study [19,20] evaluated the effect of 3–6 IL-2 cycles added to single or dual nucleoside analogue therapy administered over 1 year compared to antiretroviral therapy alone. The second study [21,22] evaluated the effect of a baseline IL-2 cycle in patients who either subsequently interrupted or continued HAART for 6 months. Biomarkers and immune parameters were analyzed pre- and post-IL-2 administration, as well as long-term at the end of each trial, in order to characterize the duration of any IL-2 effect, its relationship to HIV viremia and changes in CD4 T cell proliferation.

Methods

Clinical Trials

The methodology and primary outcomes of both these trials have been published previously [19–22]. In the first study [19] 57 chronically HIV-infected IL-2-naïve adults on nucleoside reverse transcriptase inhibitor (NRTI) mono- or combination therapy were randomized 1:1 to receive either 3–6 intermittent cycles of IL-2 plus ART or ART alone and were followed for one year. Cycles of human recombinant IL-2 (Novartis, Emeryville, CA) were administered no more frequently than every 8 weeks for 5 consecutive days as continuous infusions of 6–18 million international units per day.

The second study [21,22] consisted of 40 chronically HIV-infected IL-2-experienced adults who were on HAART therapy prior to receiving a baseline cycle of IL-2 (subcutaneous injections of 7.5 million international units twice daily). Subjects were then randomized 2:1 either to interrupt or continue HAART for 6 months, during which time additional IL-2 cycles were administered with 10 days of peri-cycle HAART, if CD4 T cell counts fell below 90% of baseline.

Participants in both trials were evaluated monthly throughout their study periods. Intracellular Ki67 expression in CD4 T cells was determined via flow cytometry at baseline in both studies, at 12 months follow-up in Study 1, and at 1 month post-HAART interruption in Study 2, as described in [20].

CRP and D-dimer measurements

Concentrations of CRP and D-dimer were determined from cryopreserved plasma collected at baseline, upon completion of the first IL-2 cycle (Day 6 in the first study and Day 5 in the second), and at the end of the study period (Month 12 in Study 1 and Month 6 in Study 2). For subjects in Study 2 who interrupted HAART, levels were also measured 1 month after the baseline IL-2 cycle.

Plasma CRP levels were determined using high-sensitivity (hsCRP) immunonephelometry (BN-II, Siemens) with a detection limit of 0.175 mg/L. D-dimer concentration was determined via latex agglutination (Liatest, Diagnostica Stago) with a detection limit of 0.20 mg/L.

Statistical Methods

Median within-group differences from baseline to either the end of the first IL-2 cycle or the entire study period were assessed via the non-parametric Wilcoxon Signed-Rank test. Between-group comparisons between randomized treatment groups within each trial were assessed via the non-parametric Mann-Whitney U test. Spearman Rank correlation tests and linear regression analysis were used to evaluate the bivariate associations between magnitude of change in hsCRP, D-dimer, CD4 T cells, and HIV-RNA plasma levels at these same time points.

Results

Participant Characteristics

Demographic characteristics and median values for baseline immune parameters and biomarkers for both treatment groups in each trial are summarized in Table 1. No thrombotic or cardiovascular serious adverse events occurred in either trial during these study periods.

Effects of IL-2 on hsCRP and D-dimer

By the end of the initial IL-2 cycle, IL-2 recipients from both trials demonstrated significant increases (all $p < 0.0001$) in median plasma levels of hsCRP (Study 1 = 138.6 mg/L, interquartile range (IQR): 87.9–173 mg/L; Study 2 = 58.9 mg/L, IQR: 35.9–84.5 mg/L) and D-dimer (Study 1 = 3.1 mg/L, IQR: 2.3–4.7 mg/L; Study 2 = 0.4 mg/L, IQR: 0.2–0.8 mg/L). However, as shown in Figure 1, these increases were transient, as hsCRP (Panels A and B) and D-dimer (Panels C and D) levels returned to baseline in IL-2 recipients from each trial by the end of their respective study periods (Figure 1). In the second trial, both hsCRP and D-dimer had returned to baseline as early as 1 month post-IL-2 administration (data not shown).

Association between Changes in hsCRP, D-dimer, and Immune Parameters

Peri-IL-2 cycle increases in hsCRP and D-dimer positively correlated among IL-2 recipients in Study 1 ($R = 0.43$, $p = 0.036$) and Study 2 ($R = 0.57$, $p = 0.0002$); however, levels of hsCRP and D-dimer were not associated at baseline in either trial. These end of IL-2 cycle increases in hsCRP and D-dimer were not correlated to baseline HIV-RNA viral load or CD4 T cell count in either study. Long-term changes (baseline to either Month 12 in Study 1 or Month 6 in Study 2) in hsCRP or D-dimer with either HIV-RNA viral load or CD4 T cell count were also not associated (data not shown). In addition, no correlations were observed between changes in CD4 T cell proliferation (Ki67 expression), hsCRP, or D-dimer levels over the same time periods in either trial (data not shown).

Discussion

This study demonstrates that IL-2 cycles cause marked transient increases in plasma levels of the acute phase reactant CRP and the thrombotic marker D-dimer, both in the setting of inadequate HIV-RNA suppression (i.e., mono or dual nucleoside analogue therapy) and with viral suppression to <50 copies/ml on HAART.

Significant associations between inflammatory biomarkers and endothelial activation markers have been demonstrated in HIV-infected patients [17], suggesting a potential mechanism by which such markers may portend cardiovascular or thrombotic disease. In addition, data from the SMART study suggested increases in biomarker levels observed following HAART interruption were associated with increased rates of non-HIV-related adverse events [18]. However, the clinical significance of the hsCRP and D-dimer elevations observed in these two trials is unclear, as these levels returned to baseline by 1 month post-IL-2 and remained at baseline when measured up to 12 months after an initial IL-2 cycle. This finding is consistent with studies in malignant melanoma patients, which demonstrated that IL-2-induced increases in several pro-coagulant markers, including thrombin-antithrombin and plasmin-antiplasmin complexes, returned to baseline within 24 hours post-IL-2 administration [11]. Moreover, no serious clinical events related to thromboses, emboli, or cardiovascular events were reported during the primary study periods in either of these trials. In contrast to findings from the SMART study [18], increased adverse clinical events were not observed among subjects in Study 2 who interrupted HAART, possibly due to the small sample size, shorter duration of follow-up (6 months), and better immune status (median CD4 T cell count = 947 cells/ μ l) compared to subjects in the SMART study.

Of note, however, a significantly greater number of serious cardiovascular events were observed in the IL-2 treatment arm compared to the placebo arm (40 versus 14 events, respectively) of the multi-center ESPRIT trial, the most common being peripheral emboli and thromboses (13 versus 2 events, respectively) [6]. Given the randomized design of the ESPRIT trial, this finding supports a causal relationship for these adverse events to IL-2. However, the timing of their clinical presentation raises questions as to the underlying pathologic mechanism.

These data offer the first randomized and controlled comparative analysis of inflammatory and thrombotic biomarkers in HIV-infected IL-2 recipients versus non-recipients, both in the presence and absence of HAART. The striking impact of IL-2 treatment on these biomarkers should warrant evaluating thrombotic and cardiovascular risk factors prior to IL-2 treatment, regardless of HIV status. With outcome data from the SILCAAT and ESPRIT trials indicating that IL-2 provides no clinical benefit in chronic HIV infection treated with HAART [5,6], the present findings suggest a possible mechanism to explain some of the adverse clinical events associated with IL-2 treatment.

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Figure 1. IL-2-associated changes in biomarker levels

Significant increases were evident immediately following interleukin (IL)-2 cycling in plasma high-sensitivity C-reactive protein (hsCRP) and D-dimer levels in Study 1 (A and C) and Study 2 (B and D), which normalized by long-term follow-up, regardless of the presence of HAART and, hence, HIV viremia. *HAART* = highly active antiretroviral treatment; *D* = day, *M* = month; * $p < 0.0001$

Table 1

Baseline characteristics, immune parameters, inflammatory, and thrombotic biomarkers. Median values are shown.

	STUDY 1 IL-2 recipients	Controls	STUDY 2
N	28	29	40
Sex (% male)	97	100	98
Age (years)	38	36.5	46
IL-2-experienced	No	No	Yes (≥ 3 cycles)
Baseline IL-2 cycle	Yes	No	Yes
Additional IL-2 cycles [*]	3–6	0	0–1
CD4 T cells/ μ l	416	371	947
HIV-RNA VL (copies/mL)	3580	5926	< 50
hsCRP (mg/L)	0.91	0.90	1.15
D-dimer (mg/L)	0.25	0.21	0.19

^{*} Range of additional IL-2 cycles given over study period; IL = interleukin; VL = viral load; hsCRP = high sensitivity; C-reactive protein