Differential dynamics of Epstein-Barr virus in individuals infected with human immunodeficiency virus-1 receiving intermittent interleukin-2 and antiretroviral therapy

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Interleukin-2 (IL-2) increases circulating CD4+ lymphocytes in patients infected with human immunodeficiency virus-1. We studied Epstein-Barr virus (EBV) dynamics in 40 patients treated with antiretroviral therapy (ART) plus different IL-2 regimens. EBV-DNA tended to increase in both peripheral blood cells and plasma after continous infusion followed by intermittent subcutaneous *high-dose* IL-2, while EBV-DNA decreased in cells (p=0.0078) and disappeared in plasma after intermittent subcutaneous *low-dose* IL-2. Over 12 months, the dynamics of EBV differed between the two groups both in cells (p=0.0184) and plasma (p=0.0114). Thus, as a function of dose, IL-2 therapy may significantly affect the dynamics of EBV infection.

Key words: EBV, HIV-1, IL-2, ART

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Several obstacles remain in defining a life-long therapeutic regimen for the treatment of human immunodeficiency virus-1 infection. In addition to simplifying antiretroviral therapy (ART), therapies that combine interleukin (IL)-2 with ART are currently being evaluated in phase III clinical trials. IL-2, a cytokine that controls several aspects of the immune response, consistently increases circulating CD4+ lymphocytes in HIV-1-infected patients, even without complete suppression of HIV-1 replication.[1-3] IL-2 has been reported to induce replication of human herpesvirus 8, the etiological agent of Kaposi's sarcoma.[4] Kaposi's sarcoma and B-cell non-Hodgkin's lymphoma are the most common malignancies in subjects infected with HIV-1. Notably, most of the non-Hodgkin's lymphomas are associated with the Epstein-Barr virus (EBV), an ubiquitous human herpesvirus that can promote B-cell lymphomagenesis in immunocompromised subjects.[5,6]

The effects of IL-2 therapy on the dynamics of EBV infection are unknown. Several IL-2-induced cytokines, such as tumor necrosis factor-α and β and IL-6, may stimulate the proliferation of B cells, thus leading to expansion of EBV-positive B cells.[7-9] Furthermore, IL-2 can directly stimulate the proliferation of B cells from HIV-1-infected individuals.[10] In addition, B-cell stimulation and EBV-DNA load were shown to increase in patients with a significant gain in CD4+ lymphocytes but incomplete suppression of HIV-1 plasma viremia during ART.[11] Because of the high incidence of HIV-1- and EBV-coinfected patients, we investigated the impact of different IL-2 therapeutic regimens on EBV-DNA load in peripheral blood mononuclear cells (PBMC) and plasma of HIV-1-infected individuals.

Design and Methods

Patients. PBMC and plasma samples were obtained from patients randomized for 12 months to the following treatment groups: group A: ART plus *civ/high-dose* IL-2 (continuous intravenous infusion (civ) of 12 million international units (MIU) IL-2/day for 2 cycles at an 8-week interval followed by subcutaneous (sc) IL-2, 7.5 MIU, twice daily for 5 days at 8-week intervals); group B: ART plus *high-dose* IL-2 (7.5 MIU sc twice daily for 5 days at 8-week intervals; group C: ART plus *low-dose* IL-2 (3 MIU sc, twice daily for 5 days at 4-week intervals; group D: ART alone. Details of the ART regimens and clinical and immunological characteristics of participants have been reported elsewhere.[2] Patients enrolled in group C showed approximately half the adverse effects associated with IL-2 toxicity than did patients enrolled in groups A or B[2]. PBMC and plasma samples, obtained at study entry (baseline) and at study completion after 12 months (post-therapy), were cryopreserved at -80°C.

Quantification of EBV-DNA. The EBV in cells and plasma was quantified by a real-time quantitative polymerase chain reaction, exactly as detailed elsewhere.[11] EBV load was expressed as EBV-DNA copies/10^5 cells. A conversion factor of 25x was used to estimate the number of EBV-DNA copies/mL of plasma.[11]

Statistical analysis. Baseline CD4+ cell counts, HIV-1 RNA plasma viremia and EBV-DNA loads in cells and plasma were com-

pared pairwise between groups by the Mann-Whitney test. Changes within groups were estimated using the Wilcoxon's signed-rank test, and between groups using the Mann-Whitney test. Both within and between group comparisons for EBV in cells and plasma were also stratified according to the immunological response of ART-treated patients. An immunological response was defined as an increase >30% from baseline in the $CD4^+$ cell count, with an absolute value >100 cells/μL.[11] Arbitrary values were attributed to plasma samples with undetectable EBV levels to include them in the statistical analyses; similar results were obtained using either 0 or 25 copies/mL as the arbitrary value. All *p* values were based on two-sided testing, and statistical analyses were carried out with SAS statistical software (Release 8.02; SAS Institute, Cary, NC, USA, USA).

Results and Discussion

At baseline, the number of $CD4^+$ cells/μL and values of HIV-1 plasma viremia were not significantly different among the four arms of the trial. All patients were positive for EBV-DNA in PBMC, with a mean viral load of 311 (range, 2-2,294) copies/10^5 cells. Eight patients also had EBV-DNA detectable in plasma (range, 59-620 copies/mL). Neither cell nor plasma EBV values differed significantly among the groups (Table 1). All patients were on stable ART based on two non-nucleoside reverse transcriptase inhibitors at study entry. A protease inhibitor was added to the pre-existing regimens at the beginning of the study.[2] Over the treatment period, HIV-1 plasma viremia decayed in most patients, but this decrease was statistically significant (p=0.0244) only in those treated with *high-dose* IL-2 (Figure 1A, panel B). In spite of persistent HIV-1 plasma viremia, the number of $CD4^+$ lymphocytes increased significantly in all IL-2-treated patients. Changes (± standard error) of $CD4^+$ cells from baseline were +681 (±153) cells/μL for the *civ/high-dose* IL-2 arm (p=0.0039), +819 (±146) cells/μL for the *high-dose* IL-2 arm (p= 0.0010), and +795 (±160) cells/μL for the *low-dose* IL-2 arm (p=0.0078) (Figure 1A, panels A, B, and C). These increases were significantly higher than those observed in patients receiving ART alone (Figure 1B), in whom $CD4^+$ lymphocytes only increased from 353 (±29) to 446 (±43) cells/μL (+93 ± 35 cells/μL) (Figure 1A, panel D). In particular, only six of 12 ART-treated patients showed a gain in $CD4^+$ lymphocytes (i.e., immunological responders); in the others, $CD4^+$ cell counts remained fairly stable or decreased (Figure 1A, panels D1 and D2). In agreement with a previous study,[11] EBV-DNA levels increased from baseline (+815±361 copies/10^5 cells; p=0.0306) in the subset of ART-treated immunological responders, while they remained fairly stable in the remaining patients (+28±76 copies/10^5 cells; p=0.687) (Figure 1A, panels D1 and D2). EBV-DNA tended to increase also in patients treated with *civ/high-dose* IL-2, although this increase was not statistically significant (+439±295 copies/10^5 cells; p=0.109) (Figure 1A, panels A and B). An opposite trend was observed in all patients treated with *low-dose* IL-2 whose EBV-DNA levels after 12 months were significantly lower than at baseline (-202±137 copies/10^5 cells; p=0.0078) (Figure 1A, panel C). This change in EBV load differed significantly from those observed in patients treated with *civ/high-dose* IL-2 (p=0.0184) and in the subset of ART-treated immunological responders (p= 0.0097) (Figure 1B).

Table 1. Baseline characteristics of HIV-1-infected patients.

	All patients	*Treatment* *ART+IL-2 civ/high dose*	*ART+IL-2 high dose*	*ART + IL-2 low dose*	*ART*
No. of individuals	40	9	11	8	12
$CD4^+$ T cells/μL mean (range)	348 (189-610)	337 (221-459)	332 (189-610)	377 (275-506)	353 (230-500)
HIV-1 RNA plasma copies/mL mean (range)	23,399 (20-181,184)	39,064 (53-181,184)	16,071 (188-52,348)	31,601 (603-134,688)	12,901 (20-50,000)
EBV-DNA copies /10^5 cells mean (range)	311 (2-2,294)	291 (2-1,669)	458 (47-2,294)	242 (2-1,353)	237 (2-1,160)
No. of individuals EBV-DNA+ (plasma) EBV-DNA copies/mL (range)	8 (59-620)	1 (63)	2 (70-93)	2 (96-139)	3 (59-620)

Only eight patients had detectable EBV-DNA in plasma at baseline, while 13 had detectable levels after 12 months. A weak correlation was found between cell-associated and plasma EBV-DNA values (Figure 2A). Consistent with the trend observed in PBMC, plasma EBV-DNA load tended to increase in patients treated with *civ/high-dose* IL-2 (p=0.0625). In contrast, all patients treated with *low-dose* IL-2, including two who were positive at baseline, tested negative for EBV-DNA in plasma after 12 months of therapy (Figure 2B). The change in plasma EBV-DNA load observed in the *civ/high-dose* IL-2 arm differed significantly from the changes observed in the *low-dose* IL-2 arm (p=0.0114) and in the subset of ART-treated non-immunological responders (p=0.0124).

None of the patients had a history of symptomatic EBV infection prior to or during the study. One subject who had received *high-dose* IL-2 developed and died of Castelman's disease 2 years after study completion, and a second subject who had received *civ/high-dose* IL-2 developed non-Hodgkin's lymphomas 4 months after termination of the study. In this study, only 50% of patients treated with ART alone showed a moderate increase in $CD4^+$ cell counts and, consistent with previous observations,[11] these patients also showed a concomitant increase in EBV-DNA load. A similar trend was observed in patients treated with *civ/high-dose* IL-2. In contrast, EBV-DNA levels decreased significantly in individuals who received *low-dose* IL-2. This opposite effect may be due to several factors. In ART-treated patients, increases in EBV load have been associated with increased immunoglobulin levels, a surrogate marker for B-cell stimulation.[11] Although the impact of

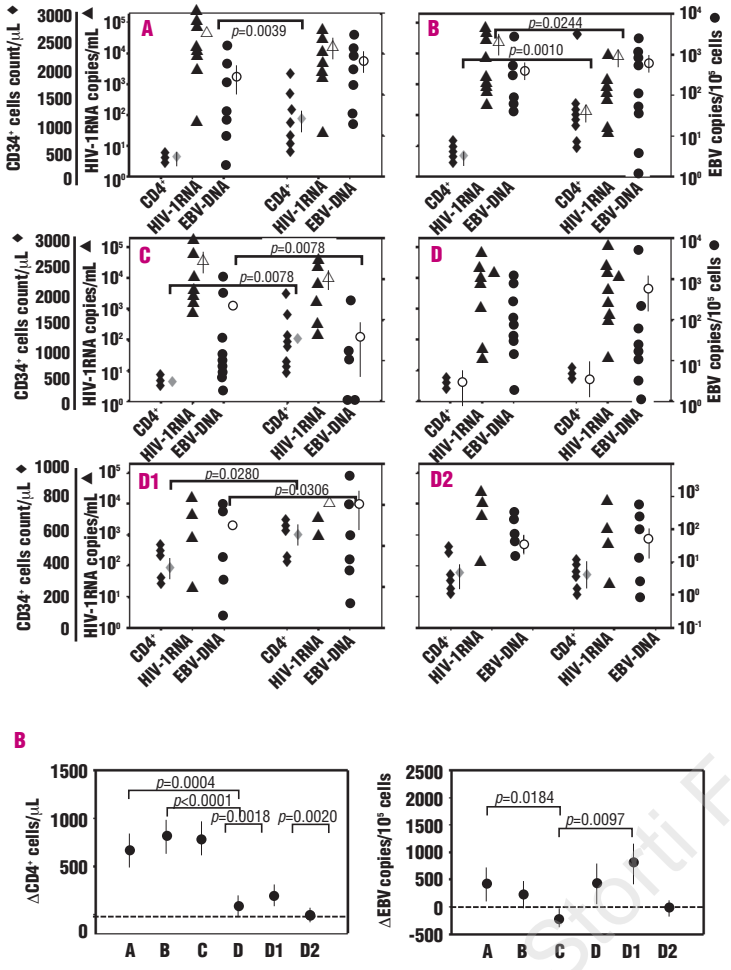
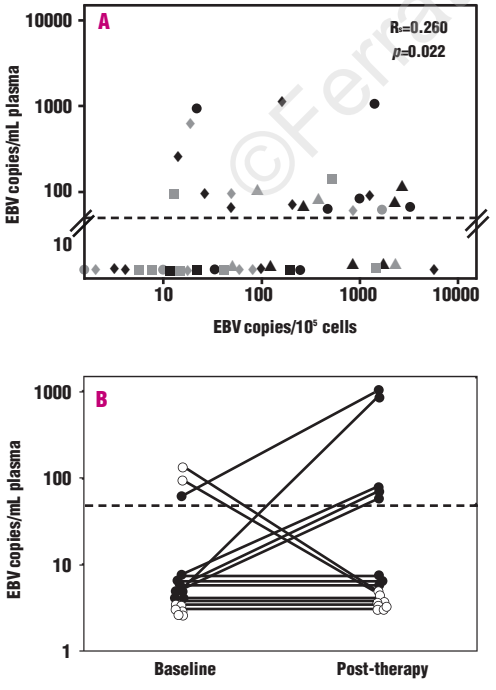


Figure 1. A. Distribution, mean and standard error (SE) of plasma HIV-1 RNA , $CD4^+$ T-cell count and cell-associated EBV-DNA load at baseline and post-therapy in individuals treated with *civ/high dose* IL-2 (A), *high dose* IL-2 (B), *low dose* IL-2 (C), or ART alone (D). Patients treated with ART alone were further divided into subgroups according to increased (D1) or not increased (D2) $CD4^+$ cell count. **B.** Change (mean and SE) from baseline to post-therapy values of $CD4^+$ cell count and cell-associated EBV-DNA load in the different groups of patients.



IL-2 on EBV expression is unknown, studies have shown that IL-2 induces a dose-dependent proliferation of B cells *in vitro*.[12] Moreover, B cells from HIV-1-infected patients express higher levels of IL-2 receptor than do B cells from normal donors, resulting in an increased IL-2 responsiveness.[10] In addition, among the IL-2-induced cytokines, tumor necrosis factor-α and β promote several B-cell functions, including cell proliferation and immunoglobulin production.[7,13] Of interest, levels of tumor necrosis factor-α were shown to increase in patients treated with high-dose IL-2, but to decrease in patients treated with low-dose IL-2.[14] Thus, dissimilar B-cell stimulation in patients receiving either low or high doses of IL-2 may account for the different EBV dynamics described here. Furthermore, while high concentrations of IL-2 may enhance production of several pro-inflammatory cytokines, via binding to low-affinity receptors expressed on NK cells,[3,15] low concentrations

Figure 2 (left). A. Relationship between plasma and cell-associated EBV-DNA load at baseline (open circles) and after 12 months (closed circles) of therapy in patients treated with *civ/high dose* IL-2 (○, ●), *high dose* IL-2(△, ▲), *low dose* IL-2 (□, ■), or ART alone (◇, ◆). B) Baseline and post-therapy values of EBV in plasma in subjects treated with *civ/high dose* IL-2 (●) and *low dose* IL-2 (○).

of IL-2 may promote expansion of cytotoxic T lymphocytes, thus restoring protective immunity against EBV, by binding to high-affinity IL-2 receptors expressed on T lymphocytes. In this regard, previous studies demonstrated that low dose IL-2 prevented the development of EBV-associated lymphoproliferative disease in mice reconstituted with PBMC from EBV-seropositive subjects, a protective effect mainly mediated by $CD8^+$ lymphocytes.[16]

Although specific studies are required to investigate the impact of IL-2 and tumor necrosis factor-α/β on B-cell stimulation and EBV expression, the present findings suggest that intermittent therapy with *low-dose* IL-2 regimens should be considered in EBV- and HIV-1-coinfected patients, particularly in those at risk of developing EBV-induced malignancies.

ADR, GP, AL, GT conceived the study, and contributed to the discussion of results; NB performed the experiments under the direction of ADR; SG and SN collected samples, and virological and immunological data of the patients; PDB performed the statistical analyses. ADR and GP wrote the manuscript with the contribution from other authors. The authors have no conflicts of interest

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