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Short communication

IL-18 and Stem Cell Factor affect hematopoietic progenitor cells in HIV-infected patients treated during primary HIV infection

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ABSTRACT

The impact of early antiretroviral therapy (ART) during Primary HIV Infection (PHI) on the hematopoietic progenitor cells (HPCs) homeostasis is not available. This study aimed to characterize HPCs and their relationship with cytokines regulating progenitors function in ART-treated patients with PHI.

We enrolled HIV infected patients treated with ART during PHI. Circulating HPCs, Lymphoid-HPCs (L-HPCs) frequency and plasmatic concentrations of IL-7, IL-18 and Stem Cell Factor (SCF) were analysed at baseline and after 6 months of therapy.

ART introduction during PHI restored the decline of L-HPCs, induced a decrease in the level of pro-inflammatory IL-18 cytokine and a parallel increase of SCF. Moreover, L-HPCs frequency positively correlated with IL-18 at baseline, and with SCF after 6 months of therapy, suggesting that different signals impact L-HPCs expansion and maintenance before and after treatment. Finally, the SCF receptor expression on HPCs decreased after early ART initiation.

These insights may open new perspectives for the evaluation of cytokine-driven L-HPCs expansion and their impact on the homeostasis of hematopoietic compartment during HIV infection.

1. Introduction

Hematopoietic progenitor cells (HPCs) participate in the response to both acute and chronic infection and play a pivotal role in determining the immune reconstitution capability during HIV infection [1–3]. Several cytokines contribute to HPCs homeostasis. Stem Cell Factor (SCF) plays a central role in the regulation of hematopoiesis and acts via CD117/c-kit receptor at multiple levels of the hematopoietic hierarchy, to promote cell survival, proliferation, differentiation, adhesion and functional activation [4]. The mechanisms leading to changes in serum SCF levels in individuals with HIV infection are unknown to date. During HIV infection, low SCF levels have been shown in more advanced stages of the disease, while elevated values have been observed in patients with asymptomatic HIV or with secondary infections [5]. SCF has a strong synergistic effect with most of the lineage-restricted cytokines: in particular, the synergistic interaction of SCF with IL-7 has been demonstrated to play an essential role in T cell development and differentiation [6]. IL-7 is well known as a lymphopoietic cytokine produced by non-hematopoietic stroma cells and is required for the development and persistence of T cells in the periphery [7]. In chronically HIV-infected patients, a strong inverse correlation has been observed between plasma IL-7 levels and CD4+ T cell numbers, as well as with CD4+ T cell reconstitution after initiation of ART [8]. The administration of r-hIL-7 led to an increase in naive and central memory CD4+ T cells related to enhanced cell proliferation and possibly due to an increased thymic output and/or cell survival [9]. Another cytokine involved in HPCs homeostasis is IL-18. It has been demonstrated that IL-18 acts in synergy with IL-7 to promote *ex vivo* expansion of T lymphoid progenitor cells (L-HPCs) [10]; moreover, in a recent paper, Silberstain and co-authors identified IL-18 as a regulator of HPCs quiescence [11]. During HIV infection, IL-18 correlates with plasma HIV-RNA and high concentrations of IL-18 are predictive of unfavourable outcomes in HIV-infected patients [12].

SCF/c-Kit signaling is important for T cell differentiation and the level of c-kit expression on HPCs has been recently associated to different functional properties. Recently Kobayashi M. and co-authors

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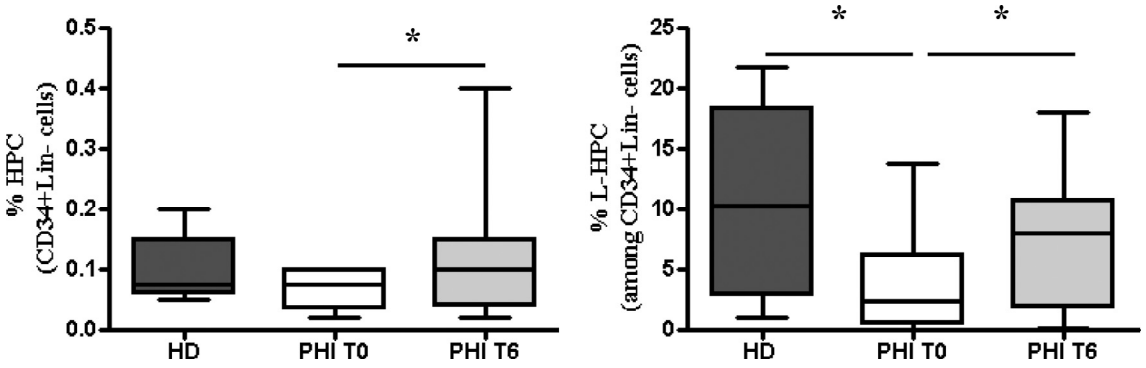

Fig. 1. Frequency of HPCs and L-HPCs in HIV-infected patients treated with ART during PHI. HPCs (left panel) and L-HPCs (right panel) frequency in HD (n = 6, grey bars) and PHI (n = 14) were evaluated by flow cytometry before (PHI T0, white bars) and after 6 months of ART (PHI T6, light grey bars). Results are shown as Box and Whiskers. The Mann-Whitney test was used for groups comparison and Wilcoxon matched paired test was applied to compare different time points. *: $p < .05$.

reported that sustaining high level of c-Kit expression by a protein tyrosine phosphatase PRL2 is crucial for T cell progenitors expansion and commitment during early stage of T cell development [13]. Moreover, other studies showed that HPCs with low levels of surface c-Kit expression exhibit enhanced self-renewal and long-term reconstitution potential, whereas HPCs with high expression levels of c-Kit show restricted self-renewal capacity [14].

Recently we showed that pro-inflammatory cytokines modulate L-HPCs frequency in both ART naïve patients with primary or chronic HIV infection [15]. Moreover, we documented a negative correlation between the pro-inflammatory cytokine microenvironment and T cell differentiation potential of bone marrow CD34+ progenitor cells during chronic HIV infection [2].

To date, no data are available on the effect of ART treatment on HPCs homeostasis during primary HIV infection (PHI). Altogether these observations prompted us to investigate the effect of ART initiated during PHI on the hematopoietic and lymphoid progenitors frequency and their relationship with plasmatic SCF, IL-7 and IL-18 levels.

2. Materials and methods

2.1. Study population

Patients with PHI (n = 14) were enrolled at the National Institute for Infectious Diseases "Lazzaro Spallanzani" (Rome). The procedures followed were in accordance with the ethical standards of the Helsinki Declaration. The Ethics Committee of the Institute approved the study protocol (ALPHA n. 68/2011 and SIREA n. 9/2014 studies) and written informed consent was obtained from all patients.

PHI patients were grouped according to Fiebig classification [16] as follows: II/III (n = 2), IV (n = 5), V/VI (n = 7). At baseline, median CD4+ cell count was 596/mm^3 (IQR, 419-719) and median Log_{10} HIV-RNA was 4.87 (IQR: 1.03-10.47) copies/ml. ART was prescribed by HIV physicians according to current National guidelines. After 6 months of ART, median CD4 + cells were 809/mm^3 (IQR, 657-945, p = .01) and HIV-RNA < 40 copies/ml was found in all participants ($p < .0001$). Healthy individuals (HD, n = 6) were used as control group. We cannot enrol a control group of PHI patients maintained 6 months without ART due to ethical reasons.

2.2. PBMC separation and flow cytometry

Peripheral blood mononuclear cells (PBMC) were isolated from blood by density gradient centrifugation (Lympholyte-H; Cederlane, Canada); plasma was stored at −80 °C for quantification of cytokines. Evaluation of HPC and L-HPC percentage was accomplished with 2×10^6 PBMC stained with anti-CD45 APC-H7, anti-CD34 APC, anti-CD45RA PE-Cy7, anti-CD10 PerCp-Cy5.5, anti-CD117 PE, anti-Lin FITC (from BD Biosciences, USA. Acquisition of 500,000 events was performed in the leukocyte-gated population on FACS CANTO II and analysed with FACS DIVA software (BD Biosciences, USA).

2.3. Quantification of plasmatic cytokines

Quantification of plasmatic IL-7, IL-18 and SCF was carried by multiplex bead-based assays Bio-Plex Pro Human (BioRad Laboratories, CA, USA) following the manufacturer's recommendations. Plates were measured using the Bio-Plex MagPix System and analysed with the Bio-Plex Manager version 6.0 (BioRad Laboratories, CA, USA).

2.4. Statistical analysis

Quantitative variables were compared among groups with non-parametric Mann–Whitney or Wilcoxon test. Spearman rank test was used to determine correlations. A p value less than .05 was considered statistically significant. Statistical analyses were performed using Prism 6.0 (Graphpad Software Inc., La Jolla, California, USA).

3. Results

3.1. Frequency of HPCs and L-HPCs in HIV-infected patients treated with ART during PHI

To evaluate the impact of early ART on HPC and L-HPC compartments, their frequency was analysed before and after 6 months of ART started during PHI by flow cytometry. HPCs were defined as CD34+ Lin-cells and L-HPCs as CD45RA+CD10+CD117- cells within CD34+Lin- population. Before ART, HPCs frequency was similar between PHI and HD (Fig. 1, left panel), while L-HPCs frequency was lower in PHI than in HD (Fig. 1 right panel). After 6 months of ART, frequency of both HPCs as well as of L-HPCs significantly increased in respect to baseline (Fig. 1).

3.2. Quantification and correlation of IL-7, IL-18 and SCF with L-HPCs frequency and expression of SCF receptor (CD117/c-kit) on HPCs in HIV-infected patients treated with ART during PHI

The effect of ART initiated during PHI on soluble mediators playing an essential role on hematopoietic homeostasis, was measured by plasmatic concentrations of IL-18, IL-7 and SCF (Fig. 2). Before ART, IL-7 and IL-18 levels were higher in PHI than in HD, while no differences were observed in SCF levels (Fig. 2A). After 6 months of ART, the level of IL-18 significantly declined compared to pre-treatment values and returned comparable to those observed in HD; a similar effect was observed also for IL-7, although not reaching the statistical significance. Moreover after 6 months of ART, a significant increase of SCF when