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Short Communication

HIV-1 Tat is present in the serum of people living with HIV-1 despite viral suppression

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ABSTRACT

Objectives: Despite successful human immunodeficiency virus (HIV) control with combination antiretroviral therapy (cART), individuals with HIV still face health risks, including cancers, cardiovascular and neurocognitive diseases. An HIV protein, Tat, is potentially involved in these HIV-related diseases. Previous studies demonstrated circulating Tat in the blood of untreated people with HIV. Here, we measured Tat levels in the serum of cART-treated people with HIV to examine the effect of cART on Tat production. **Methods:** Serum samples from 63 HIV-positive and 20 HIV-seronegative individuals were analyzed using an ELISA assay that detected Tat concentrations above 2.5 ng/mL.

Results: Among HIV-positive individuals, the Tat level ranged from 0 to 14 ng/mL. 25.4% (16 out of 63) exceeded the 2.5 ng/mL cut-off, with a median HIV Tat level of 4.518 [3.329–8.120] ng/mL. No correlation was revealed between Tat levels and CD4+ T cell counts, serum HIV RNA, p24 antigen, or anti-Tat levels. **Conclusions:** Despite cART, circulating HIV Tat persists and may contribute to HIV-related diseases. This emphasizes the need for further research on the mechanisms of Tat action in non-infected cells where it can penetrate upon circulation in the blood.

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Introduction

Human immunodeficiency virus (HIV) infection affects ~38.4 million people worldwide (<http://www.who.int/hiv/en/>). The implementation of combination antiretroviral therapy (cART) largely decreased mortality and prolonged the lifespan of individuals with HIV [1]. However, people with HIV still face increased risks of different comorbidities. cART effectively controls viral replication but does not eliminate latently infected cells, posing challenges in achieving a cure [1].

HIV Tat is needed for viral transcription and pathogenesis [2]. Beyond its classical role in transcriptional activation, Tat is efficiently secreted from HIV-infected cells and can penetrate non-

infected cells [2]. Emerging evidence suggests that Tat is involved in various aspects of HIV-associated comorbidities [2,3]. Tat circulates in the blood of people with HIV [3–6]. Pre-cART era reports indicated Tat serum concentrations from 0.1 to 40 ng/mL [4,5]. In individuals with low to undetectable viral loads, one study reported high serum Tat levels, in the 250–550 ng/mL range [6], while we observed 0.2–9 ng/mL serum Tat [3]. To clarify this issue, this prospective multicentre study aimed to quantify Tat serum concentrations in a larger cohort of cART-treated people with HIV using a quantitative enzyme-linked immunosorbent assay (ELISA).

Materials and methods

Blood collection

Blood samples from individuals who signed a written informed consent were collected at the Hôpital Saint-Louis, Paris, or Mont-

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pellier University Hospital Center, Montpellier (agreements 2016-A01316–45 [3], 14/EFS/033, VIHEBV and 233–2018-SR).

ELISA

Tat concentration was determined by ELISA as described previously [7], with minor modifications. Tat concentrations above 2.5 ng/mL were detected. HIV-1 p24 concentration was determined using an HIV-antigen mAb ELISA kit (Innotest, cat.#80,564), following the manufacturer's instructions, detecting p24 concentrations above 5 pg/mL.

Statistical analysis

Tat and p24 concentrations were calculated from linear standard curves using reference dilutions. Data are presented as individual values, median [interquartile range].

Results

This study used serum samples from cART-treated people with HIV ($n = 63$) and HIV-seronegative individuals ($n = 20$). Most individuals with HIV had viral levels <20 HIV RNA copies/mL (Table 1). To confirm this lack of circulating virus, we performed a p24 ELISA test. Capsid protein p24 is a viral antigen, widely used to assess HIV infection. Only one HIV-positive individual (1.6%) had detectable p24 antigen in the serum (5.91 pg/mL) (Figure 1A), confirming that the majority of the cohort achieved effective viral suppression. As expected, HIV-seronegative individuals showed no detectable p24 antigen in their serum (Figure 1A).

To establish a specificity cut-off for the Tat ELISA, we analyzed the serum samples from HIV-seronegative individuals (Figure 1B). In this group, 10 (50%) subjects had undetectable Tat levels (0 ng/mL), as expected. For 10 others, the range of background Tat concentrations spanned from 0.280 to 2.290 ng/mL. Tat median concentration in this group was 0.140 [0–1.531] ng/mL. Based on these values, a specificity cut-off of 2.5 ng/mL was chosen for the test.

In the HIV-positive group, the median concentration of circulating Tat was found to be 0.059 [0–2.970] ng/mL, ranging from 0 (for 31 subjects) to 14.00 ng/mL (Figure 1B). Among the HIV-positive individuals, 16 (25.4%) displayed Tat concentrations above the established specificity cut-off (>2.5 ng/mL). The median concentration of Tat in this subgroup was 4.518 [3.329–8.120] ng/mL, with a minimum of 2.97 ng/mL and a maximum of 14.00 ng/mL.

Table 1

Demographic and clinical characteristics of HIV-positive individuals enrolled in the study.

Characteristic	HIV-positive individuals ($n = 63$)
Age	56 [48–77]
Female	17 (27%)
Male	46 (73%)
CD4+ T cell count/ μ L in plasma	535.5 [423.5–754.5]
HIV viral load copies/mL	
NA	7 (11%)
<20	46 (73%)
>20	10 (16%); 93.50 [48.50–69.731]*

NA, not available.

Data are presented as n (%) or median [interquartile range].

* Only four individuals had viremia above 150 HIV RNA copies/mL (Figure 1D).

The individual showing a high p24 level in the serum had a low level of circulating Tat. No sex-specific difference in Tat levels was found (Supplementary Figure 1). A weak negative correlation was observed between levels of circulating Tat and age (Figure 1C). Moreover, no association between levels of circulating Tat and either CD4+ T cell concentration or HIV RNA serum levels was found (Figure 1D–1F). Weak positive correlations between levels of circulating Tat and the percentage of CD4+ T cells and CD4+/CD8+ T cell ratio, but not the percentage of CD8+ T cells were found (Figure 1G–1I). A stepwise multiple regression model showed a significant relationship between Tat levels and sex and age ($P = 0.041$ and $P = 0.0004$, Supplementary Table 1).

Discussion

The present study investigated the presence and concentration of circulating HIV Tat protein in the serum of cART-treated individuals with HIV ($n = 63$). The majority of individuals with HIV had low levels of HIV replication (<20 HIV RNA copies/mL, Table 1). We found that 16/63 (25.4%) people living with HIV displayed Tat concentrations above the established specificity cut-off (>2.5 ng/mL) with a median concentration of 4.518 [3.329–8.120] ng Tat/mL (Figure 1B). Tat serum level was not influenced by immune suppression, as assessed by CD4+ T cell counts, or HIV replication, as assessed by HIV RNA and p24 blood levels (Figure 1C–1F). These findings thus suggest that, despite the successful control of viral replication and restoration of immune function through cART, Tat is still being produced.

The chronic secretion of Tat may play a contributory role in the development of HIV-associated pathologies. Notably, even low Tat concentrations (10–15 ng/mL, in the range of those observed in the sera of people with HIV (Figure 1B)) produce significant biological effects, e.g., DNA damage and relocalization in B cells [3].

The detected serum Tat concentrations align with the reports before cART (0.1–10 ng/mL) [4], during early cART (2–40 ng/mL) [5] or late cART era (0.2–9.0 ng/mL) [3]. We found that $\sim 25\%$ of people with HIV had detectable serum Tat levels, similar to the $\sim 29\%$ reported by Westendorp et al. [4]. Moreover, Tat concentrations in the same range (0.2–6.5 ng/mL) were reported in the cerebrospinal fluid of 37% of people with HIV who were virologically controlled with long-term cART [8]. Altogether, these observations suggest that cART and viral suppression do not significantly affect Tat production. The observed prevalence of circulating Tat also aligns with reported rates of anti-Tat antibody-mediated immune responses in people with HIV ($\sim 20\%$) [9].

Poggi et al. [6] reported notably high Tat levels in the sera of both untreated and cART-treated people with HIV, ranging from 150 to 550 ng Tat/mL. The difference with our study could stem from variations in assay specificities, sensitivities, and the studied population. Furthermore, our ELISA used a monoclonal antibody targeting the N-terminus of Tat from the most prevalent HIV-1 B-subtype (HXB2). This region, while relatively conserved, may vary across viral subtypes [10], potentially causing our assay to underestimate Tat levels in individuals with other viral subtypes.

Together with data from the pre-ART period [4], our findings suggest that Tat circulation in people with HIV is unlikely affected by cART. Circulating Tat might originate from several sources, including productively infected cells in anatomic reservoirs and latently infected cells, which are not entirely transcriptionally silent and are capable of producing viral proteins [1].

In conclusion, our study demonstrated the presence of circulating Tat in cART-treated individuals. It highlights the need for research on Tat implications in HIV-associated clinical disorders.

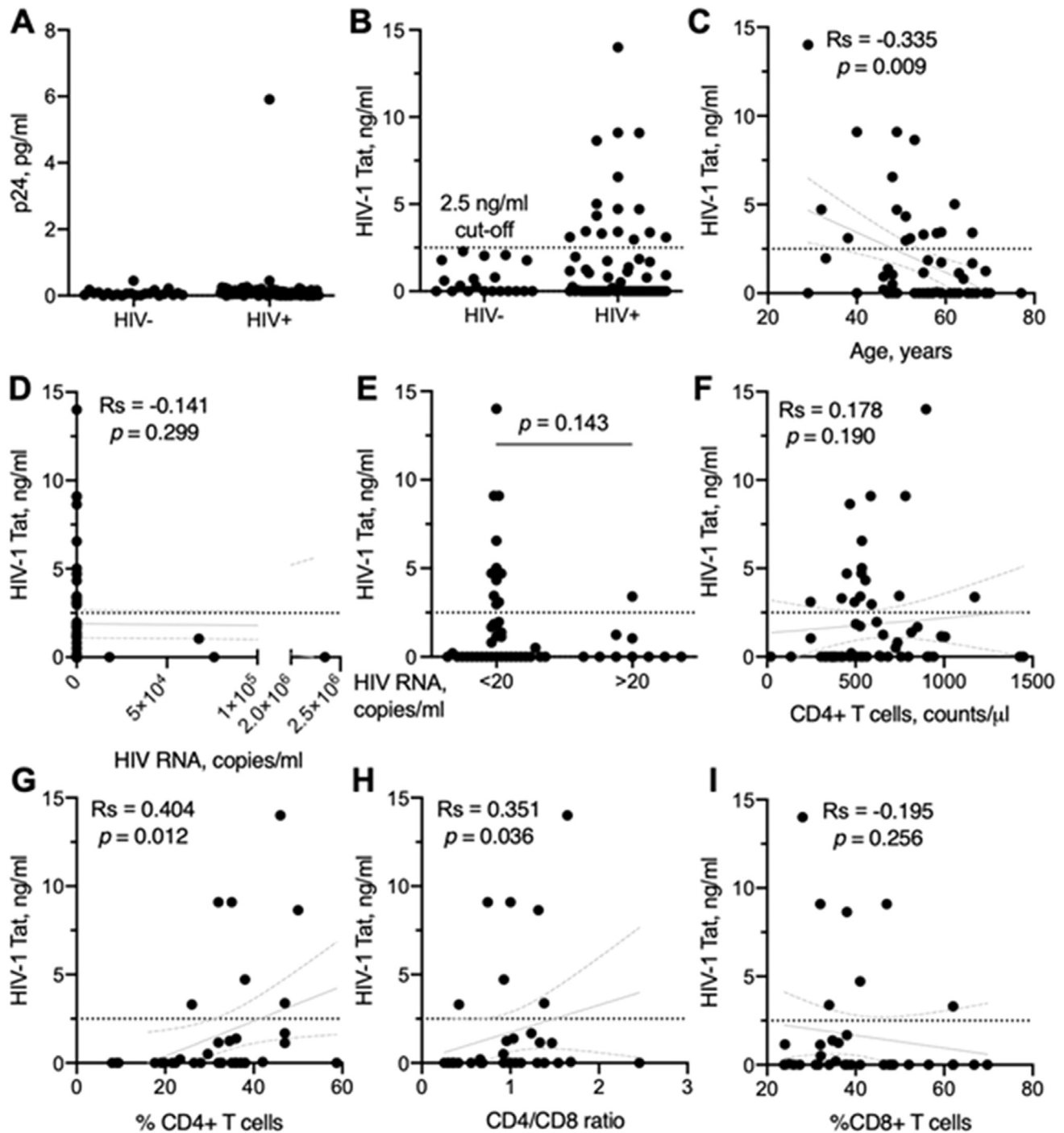


Figure 1. HIV-1 Tat and p24 antigen levels in the serum of cART-treated HIV-positive individuals. (A) Serum levels of capsid protein p24 were assessed using ELISA in cART-treated people living with HIV (HIV+, $n = 63$) and HIV-seronegative people (HIV-, $n = 20$). (B) HIV-1 Tat serum levels were measured in the same two cohorts using ELISA. An established specificity cut-off of 2.5 ng/ml is shown with a black dotted line. Data are presented as individual values. (C) Correlation between age and HIV-1 Tat serum levels in HIV-positive individuals; the gray solid line represents a linear regression and the gray dashed lines represent its 95% confidence intervals. The Spearman correlation coefficient (Rs) and its corresponding P -value are shown. An established specificity cut-off of 2.5 ng/mL is shown with a black dotted line. (D) Correlation between HIV RNA levels and HIV-1 Tat serum levels in HIV-positive individuals; the gray solid line represents a linear regression and the gray dashed lines represent its 95% confidence intervals. The Spearman correlation coefficient (Rs) and its corresponding P -value are shown. An established specificity cut-off of 2.5 ng/mL is shown with a black dotted line. (E) HIV-1 Tat protein serum levels in HIV-positive individuals with HIV RNA below and above 20 copies/mL. The P -value of the Mann-Whitney test is shown. (F) Correlation between CD4+ T cell counts and HIV-1 Tat serum levels in HIV-positive individuals; the gray solid line represents a linear regression and the gray dashed lines represent its 95% confidence intervals. The Spearman correlation coefficient (Rs) and its corresponding P -value are shown. An established specificity cut-off of 2.5 ng/mL is shown with a black dotted line. (G) Correlation between the percentage of CD4+ T cells and HIV-1 Tat serum levels in HIV-positive individuals; the gray solid line represents a linear regression and the gray dashed lines represent its 95% confidence intervals. The Spearman correlation coefficient (Rs) and its corresponding P -value are shown. An established specificity cut-off of 2.5 ng/mL is shown with a black dotted line. (H) Correlation between the CD4/CD8 T cell ratio and HIV-1 Tat serum levels in HIV-positive individuals; the gray solid line represents a linear regression and the gray dashed lines represent its 95% confidence intervals. The Spearman correlation coefficient (Rs) and its corresponding P -value are shown. An established specificity cut-off of 2.5 ng/mL is shown with a black dotted line. (I) Correlation between the percentage of CD8+ T cells and HIV-1 Tat serum levels in HIV-positive individuals; the gray solid line represents a linear regression and the gray dashed lines represent its 95% confidence intervals. The Spearman correlation coefficient (Rs) and its corresponding P -value are shown. An established specificity cut-off of 2.5 ng/mL is shown with a black dotted line. cART, combination antiretroviral therapy, ELISA, enzyme-linked immunosorbent assay.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

AS, YV and BB conceived and designed the study; AS, IT, YK, AR, DG, and BB carried out experiments; AS, YV, BB analyzed the data; LG, DB, EO, ET contributed data and materials; AS, YV, BB wrote the paper.

Ethical approval

Blood samples from HIV-positive individuals who signed written informed consent for the study and were treated at the Department of Clinical Immunology, Hôpital Saint-Louis, Paris, France, or Montpellier CHU, Montpellier, France, were collected in accordance with French legislation (agreements 2016-A01316–45, VIHEBV for Paris and 233–2018-SR for Montpellier). Whole blood samples from anonymous healthy donors of different sex and age were obtained from the “Etablissement Français du Sang”, either at the Saint-Louis Hospital, Paris, France or at Montpellier University Hospital Center (CHU), in accordance with the French legislation (agreements 14/EFS/033 for Paris and 233–2018-SR for Montpellier).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ijid.2024.106994](https://doi.org/10.1016/j.ijid.2024.106994).

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