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Effect of Antiretroviral Therapy on HIV Reservoirs in Elite Controllers

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ABSTRACT

Elite controllers suppress HIV viremia to below the limit of detection in the absence of antiretroviral therapy (ART). However, precise frequencies of $CD4^+$ T cells carrying replication-competent HIV and/or the dynamics of the infectious viral reservoirs in response to initiation and discontinuation of ART in elite controllers are unknown. We show that the size of the pool of $CD4^+$ T cells harboring infectious HIV diminished significantly following initiation of ART and rebounded to baseline upon cessation of therapy. Our data provide compelling evidence that persistent viral replication occurs in untreated elite controllers even in the absence of detectable plasma viremia.

INTRODUCTION

Antiretroviral therapy (ART) suppresses plasma viremia to below the limit of detection in the vast majority of human immunodeficiency virus (HIV)-infected individuals and reduces the rate of viral transmission, morbidity, and mortality where antiretroviral drugs are available [1]. However, standard ART alone cannot eradicate HIV in infected individuals due in part to the persistence of viral reservoirs in the peripheral blood, lymphoid tissues, and other sequestered sites [2]. Consequently, there is a growing interest in developing therapeutic strategies to eliminate persistent HIV reservoirs and/or to enhance host immunity against the virus in order to control viral replication in the absence of ART [2]. In this regard, it has been shown that a small proportion of HIV-infected individuals spontaneously control plasma viremia in the absence of ART (controllers) [3]. Moreover, a subset of such individuals (elite controllers) are capable of maintaining relatively normal $CD4^+$ T cell counts and undetectable levels (<50 copies of HIV RNA/ml) of plasma viremia for years to decades without the need for ART [3]. Although previous studies have suggested that ongoing viral replication may occur in elite controllers based on persistence of residual plasma viremia and immune activation [4, 5] as well as evidence for viral evolution in plasma HIV [6, 7], precise frequencies of infected $CD4^+$ T cells carrying replication-competent virus and/or the impact of ART on immunologic and virologic parameters have not been fully delineated in this patient population. Given recent interest in developing therapeutic strategies aimed at achieving containment of HIV replication in the absence of ART, as observed in elite controllers, and considering that a certain percentage of these individuals may eventually experience disease progression [8], we conducted the present study to address these questions.

METHODS

Isolation of CD4$^+$ T cells in peripheral blood. Peripheral blood mononuclear cells (PBMCs) were obtained from blood draw and leukapheresis from the study participants (Table 1) in accordance with protocols approved by the Institutional Review Boards of the University of Toronto, Toronto, Canada and by the Office of Human Subjects Research at the National Institutes of Health. CD4$^+$ T cells were isolated from PBMCs of HIV-infected individuals using a cell separation technique (StemCell Technologies).

Determination of plasma viremia in infected individuals receiving antiretroviral therapy. During the study period, plasma viral loads were determined using Cobas Ampliprep/Cobas Taqman HIV-1 Test Version 2.0 (Roche Diagnostics) in quadruplicate. The published limit of detection for this system is 20 copies of HIV RNA/ml of plasma. The copy number of HIV RNA below 20 copies was determined as described in Supplemental Methods.

Determination of infectious HIV burden using quantitative coculture assays. In order to determine the frequency of CD4$^+$ T cells carrying replication-competent HIV, standard and high input quantitative coculture assays were carried out as described in Supplemental Methods. The half-life of CD4$^+$ T cells carrying replication-competent HIV was determined by dividing $-\log_{10}2$ by the slope of the $\log_{10}$-transformed infectious units per million cells obtained at baseline month 6, and month 9.

Quantitative real-time PCR for measurements of HIV DNA. The frequency of CD4$^+$ T cells carrying HIV DNA was determined as described in Supplemental Methods.

Levels of immune activation in blood and sigmoid colon. The level of cellular activation was determined as described in Supplemental Methods.

Measurements of HIV-specific CD8$^+$ T cells. The frequency of HIV-specific CD8$^+$ T cells was determined as described in Supplemental Methods.

RESULTS

Three elite controllers (Subjects 1, 2, and 3) and one controller (Subject 4) (Table 1) received standard ART consisting of tenofovir/emtricitabine and raltegravir for 9 months followed by discontinuation of ART. For this study, controllers and elite controllers are defined by the ability to suppress HIV plasma viremia <500 and <50 copies of HIV RNA/ml, respectively, in the absence of ART with no more than 1 viral blip above these cut-off values (Table 1). Blood draws and leukapheresis were conducted at month 0 (prior to the initiation of ART), 6, and 9 months following the initiation of ART, and 3 months following discontinuation of ART. Gut biopsies (sigmoid colon) were also obtained at month 0 and 6 and 3 months after cessation of ART. CD4$^+$ and CD8$^+$ T cell percentages in the blood of all study subjects remained stable throughout the course of the study (Figure 1A). All elite controllers maintained <20 copies of HIV RNA per ml of plasma throughout the entire study period (Figure 1B). There was no significant difference in residual plasma viremia (1-19 copies of HIV RNA/ml) prior to and following initiation and after discontinuation of ART in the elite controllers (data not shown). In Subject 4, the baseline plasma viremia (88.0±25.9; mean±SD) became undetectable shortly after initiation of ART and returned to a detectable level (131.5±24.6; mean±SD) following

discontinuation of therapy (Figure 1B). Given that the vast majority of HIV-infected $CD4^+$ T cells carry defective virus, we conducted quantitative coculture assays to determine the frequency of cells carrying replication-competent HIV. When standard quantitative coculture assays (up to $1x10^6$ cells) were used, no replication-competent HIV could be detected from the $CD4^+$ T cells of the elite controllers. Subsequently, large numbers of $CD4^+$ T cells (37-92 replicates of $10x10^6$ cells per well, Online Table 1) of the elite controllers were subjected to high input quantitative coculture assays in order to determine the frequency of cells that could give rise to replication-competent virus. A dramatic decrease (>1 log) in the frequency of $CD4^+$ T cells carrying replication-competent HIV was seen in 2 elite controllers (Subject 2 and 3) and 1 controller (Subject 4) following initiation of ART (Figure 1C and Online Table 1). In Subject 1, the reduction in the frequency of $CD4^+$ T cells carrying replication-competent HIV at baseline versus month 6 or month 9 was modest due in part to the fact that the baseline infectious HIV burden was extremely low in this subject and detecting a larger difference post ART would have required a prohibitive number of cells. The level of replication-competent HIV was below the limit of detection in all study subjects during ART despite culturing extraordinarily large numbers of cells in replicate (average of 62 wells containing $10x10^6$ cells per well). In contrast to Subject 1, the cultures from Subject 2, 3, and 4 had readily detectable levels of replication-competent HIV at baseline and three months following cessation of ART (Online Table 1). Of note, the infectious HIV burden returned to the pre-therapy level in all study subjects 3 months following discontinuation of ART (Figure 1C and Online Table 1). Although testing for statistical significance was not performed due to the small sample size, the mean $\log_{10}$ decrease (month 0 vs. month 9) and increase (month 9 vs. month 12) of the infectious HIV burden were -1.2938 and +1.3379, respectively, clearly demonstrating that ART had an impact on the size of

the pool of infected $CD4^+$ T cells carrying replication-competent HIV in the study subjects. The half-lives of the HIV reservoir during the treatment period (month 0, 6, and 9) in Subject 1, 2, 3 and 4 were 16.93, 2.06, 2.12, and 1.01 months, respectively. Of note, short half-lives (<4 months) of the viral reservoir had been reported in previous studies involving HIV-infected individuals who initiated ART during the acute/early phase of infection [9]. Frequencies of $CD4^+$ T cells in the blood carrying HIV DNA were measured but no significant changes were observed in the elite controllers (Online Figure 1), possibly due to the fact that the vast majority of infected $CD4^+$ T cells carry replication-defective HIV. In addition, levels of immune activation (T cells expressing CD38 and HLA-DR) were evaluated in the blood and sigmoid colon and found to be decreased during ART and returning to pre-ART levels following discontinuation of therapy in three of the four subjects studied (Online Figure 2). Finally, we investigated the impact of ART on the frequency of HIV-specific $CD8^+$ T cells in the blood of the three elite controllers. As shown in Figure 1D, the frequency of HIV-specific $CD8^+$ T cells expressing intracellular IFN-γ and MIP-1ß decreased during the treatment period and levels remained low to undetectable following cessation of ART in all three individuals.

DISCUSSION

Despite the remarkable success of ART in controlling plasma viremia and recent advances in our understanding of the pathogenesis of HIV, the prospect for eradication of HIV by standard ART alone remains challenging [2]. Given the considerable difficulties of achieving a complete eradication (sterilizing cure) of HIV in a large proportion of infected individuals, immune-based strategies aimed at achieving containment of viral replication in infected individuals in the absence of ART represent more realistic and feasible avenues. In this regard, the unique ability

of elite controllers to suppress plasma viremia and maintain normal $CD4^+$ T cell counts for years to decades in the absence of ART has served as a model for achieving a 'functional' cure. To this end, it is critical to understand the dynamics of HIV reservoirs and the interplay between the virus and the host immune system in elite controllers. In the present study, we used the modulation of virologic and immunologic parameters by ART to provide evidence that on-going/residual HIV replication does, in fact, occur in individuals who had been controlling their plasma viremia in the absence of ART. We demonstrated that a short course of ART dramatically decreased the infectious HIV burden in elite controllers, providing compelling evidence that residual viral replication occurs in such individuals in the absence of therapy. Recent studies have suggested that on-going/residual HIV replication may be absent in infected individuals receiving ART as long as undetectable levels of plasma viremia (<50 copies) are maintained [10], although other studies have suggested otherwise [11, 12]. In addition, it has been suggested that residual plasma viremia fluctuates over time in elite controllers [4, 5, 13] although the clinical significance of such events and residual plasma viremia in general have not been fully delineated to date. Of note, previous studies have shown that initiation of ART in elite controllers led to decreases in the level of immune activation [14] and increases in $CD4^+$ T cell counts [15] although the size of the pool of $CD4^+$ T cells carrying replication-competent HIV was not determined in these studies. Our data suggest that on-going/residual viral replication can occur in infected individuals in the absence of detectable plasma viremia and indicate that one virologic marker (plasma viremia) alone may not accurately reflect the dynamics of HIV replication *in vivo*. While, the mechanism by which ART reduced the frequency of $CD4^+$ T cells carrying replication-competent HIV is unclear, it is conceivable that the presence of ART inhibited bystander $CD4^+$ T cell infection and/or induced transient lymphocyte redistribution.

Interestingly, the infectious HIV burden in the $CD4^+$ T cell compartment of the elite controllers rebounded back to their original baseline levels upon cessation of ART, an indication that a virologic 'set-point' exists even at this very low level of viral replication, and that host immunity can efficiently control clinically relevant plasma viremia without completely blocking viral replication or completely eliminating the infected $CD4^+$ T cells that are the source of this residual viral replication. We also demonstrated that the level of HIV-specific $CD8^+$ T cells in elite controllers gradually declined upon initiation of ART, further supporting the virologic evidence that low levels of viral replication occur in these infected individuals. It is unclear why the levels of HIV-specific $CD8^+$ T cells did not return to baseline within the observed time frame following discontinuation of therapy although possible explanations include elimination of viral antigens during the period of ART and/or bystander cellular activation of $CD8^+$ T cells after cessation of therapy. Long-term immunologic and virologic studies involving larger numbers of elite controllers, including non-HLA-$B5701^+$ individuals should be conducted to strengthen and confirm our findings.

In summary, we demonstrated that ART results in a marked diminution of the number of infected $CD4^+$ T cells carrying replication-competent HIV in elite controllers, suggesting that low levels of ongoing viral replication contributes to the maintenance of HIV reservoirs in the absence of detectable plasma viremia.

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POTENTIAL CONFLICT OF INTEREST

None declared.

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Table 1. Profiles of HIV-infected study participants

Subject	Duration of infection (years)	Number of episodes of detectable plasma viremia (highest viral load)[1]	Duration of undetectable plasma viremia[1] (years)	HLA B5701 present	CD4 count at the time of study	CD8 count at the time of study	Plasma viremia at the time of study[2]	Half-life of infected $CD4^+$ T cells (months)
1	5	0	5	Yes	670	490	<20	16.91
2	23	0	14	Yes	650	550	<20	2.06
3	14	1 (51)	9	Yes	980	710	<20	2.12
4	23	8 (372)	NA	No	560	660	88	1.01

[1]measured by a branched DNA assay with a detection limit of 50 copies per ml of plasma.
[2]measured by Cobas Ampliprep/Cobas Taqman HIV-1 Test Version 2.0 with a detection limit of 20 copies per ml of plasma.

Figure Legends

Figure 1. Levels of $CD4^+$ and $CD8^+$ T cells (A), plasma viremia (B), $CD4^+$ T cells carrying replication-competent HIV (C), and HIV-specific $CD8^+$ T cells (D) in infected study subjects following initiation and discontinuation of ART. (B) Plasma viremia was determined using Cobas Ampliprep/Cobas Taqman HIV-1 Test Version 2.0 (Roche Diagnostics) with a detection limit of 20 copies per ml of plasma. (C) The open squares represent values below the limit of detection. When cocultures were negative, the frequency was estimated to be lower than the number calculated based on the assumption that one well containing 10 million cells would be culture-positive by p24 ELISA. (D) PBMCs were incubated with overlapping 15-mer HIV Gag peptides for 6 hours and the frequency of $CD8^+CD45RO^+CD27^+$ T cells expressing intracellular IFN-γ, IL-2, MIP-1β, and TNF-α was determined by flow cytometry. The shaded area represents the period of ART