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Single-Copy Assay Quantification of HIV-1 RNA in Paired Cerebrospinal Fluid and Plasma Samples from Elite Controllers

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Abstract

Objective—Elite controllers (ECs) are a rare subset of HIV-1 infected individuals who maintain HIV-1 RNA concentrations in plasma below the lower limit of quantification of clinical assays (<20–50 copies/mL) in the absence of antiretroviral therapy. Here we examine to what extent ECs also control infection of the central nervous system (CNS).

Design—We analyzed paired cerebrospinal fluid (CSF) and plasma samples using a highly sensitive assay for HIV-1 RNA quantification.

Methods—We analyzed 28 CSF samples and 27 concurrent plasma samples from 14 ECs with the highly sensitive single-copy assay (SCA) that allows for HIV-1 RNA quantification down to less than 1 copy of HIV-1 RNA/mL.

Results—Three samples were excluded because of internal standard failure. HIV-1 RNA was detected in only 5/26 CSF samples compared to 14/26 plasma samples (P=0.02), with a median of 0.2 (range 0.1–6) copies/mL in CSF compared to 0.8 (range 0.1–189) in plasma (P<0.0001).

Conclusions—HIV-1 RNA could not be detected in CSF in most ECs using the highly sensitive SCA, and when detected it was at significantly lower frequencies and concentrations than in plasma. ECs thus control HIV-1 in the CNS very well. Whether the infrequent and small amounts

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RWP and SP designed the study. BS, RWP, SS and oversaw and characterized the study subject cohort. EL, JP, RWP and SS collected samples and interviewed subjects. VD analyzed samples. VD and SP analyzed data. BS, RWP, SP, SS and VD wrote manuscript.

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Potential conflicts of interest

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of HIV-1 RNA in the CSF reflect production from a local reservoir or virion exchange between the blood and the CSF is uncertain.

Keywords

HIV; elite controllers; cerebrospinal fluid; CSF; central nervous system; CNS; eradication

Introduction

Elite controllers (ECs) are a rare subset (less than 1%) of HIV-1 infected individuals who maintain HIV-1 RNA concentrations in plasma below the lower limit of quantification of assays approved for clinical use (20–50 copies/mL) in the absence of antiretroviral therapy (ART) [1, 2]. In some cases individuals classified as ECs are infected with a defective virus [3, 4] but most ECs are infected with strains of HIV-1 which do not contain any obvious genetic defects [5]. This is also reflected in the fact that replication-competent virus can be isolated and cultured from ECs [6–8]. Certain HLA class I alleles as well as CD4+ and CD8+ T-cell immune responses are associated to virus control whereas humoral immunity does not appear to be a major mechanism behind virus control in ECs. No single factor however seems to be completely protective nor is there one that is strictly required [9]. Despite the low HIV-1 RNA concentrations evolution occurs over time suggesting that ongoing viral replication occurs albeit at a low level in these individuals [10, 11]. It has been suggested that studies of ECs could guide the design of HIV-1 vaccines and HIV-1 eradication strategies [12, 13]. HIV-1 eradication strategies need to take into account infected cells in other compartments of the body than the blood where HIV-1 persists.

We have previously demonstrated that HIV-1 RNA can be detected in the cerebrospinal fluid (CSF) in subjects on suppressive antiretroviral therapy (ART) and that these levels are not affected by treatment intensification [14]. Therefore the central nervous system (CNS) could be a compartment that needs to be taken into account when designing HIV-1 eradication strategies. If HIV-1 vaccines and/or eradication strategies are to be based on the same mechanisms that ECs use for viral control, it is of interest how and to which extent ECs control HIV-1 infection of the CNS. We have reported that ECs control infection of the CNS as reflected in having CSF HIV-1 RNA concentrations below 2.5 copies/mL [15]. ECs also maintain CSF white blood cell counts, CSF:serum albumin ratios, and CSF neopterin, MCP-1 and IP-10 concentrations similar to HIV-1 uninfected individuals and HIV-1 infected individuals on suppressive therapy [15]. To determine if HIV-1 RNA can be found in the CSF of ECs at levels lower than 2.5 copies/mL we analyzed paired CSF and plasma samples from several time points in 14 ECs using the single-copy assay (SCA). SCA is a very sensitive assay that allows HIV-1 quantification down to 0.3 copies of HIV-1 RNA/mL in both CSF and plasma [16].

Methods

Study participants

We analyzed CSF and plasma samples from 14 individuals (of whom 8 were the same as in our previous report [15]) classified as ECs since they had, over a period of more than 12

months, three or more longitudinal plasma HIV-1 RNA determinations below the lower level of quantification of the assay used in the clinical setting in the absence of ART (<40 copies/mL). Informed consent was obtained from all study participants, and CSF was obtained solely for study purposes with concurrent phlebotomy in the context of protocols approved by the University of California San Francisco (UCSF) Committee on Human Research. Permission for analyzing the samples was also obtained by the regional ethical review board in Stockholm, Sweden.

Laboratory measurements

SCA has been described elsewhere [16] but in brief, up to 8 mL of CSF or plasma, with a known amount of RCAS (an avian retrovirus) added as an internal standard, was ultracentrifuged and viral RNA was extracted from the pelleted virions and subjected to complementary DNA synthesis followed by real-time polymerase chain reaction amplification of a 79-base pair region of HIV-1 Gag or a portion of the RCAS genome. HIV-1 RNA levels were determined using a standard curve constructed with HIV-1 of known RNA copy number. To ensure that the extraction process was successful, the level of RCAS was measured using a separate standard curve constructed with RCAS of known RNA copy number. HIV-1 RNA results by SCA each represent the median of triplicate determinations.

Since sample volume affects the lower-limit of quantification in SCA and our sample volumes varied, the lower limit of quantification also varied. We therefore, as previously described [14], assigned a value of 0.1 copy below the limit of detection for negative samples to be able to compare HIV-1 RNA concentrations in CSF and plasma.

Statistical analysis

Statistical analysis was performed using Prism (Version 5.04 GraphPad, La Jolla, California, USA). Proportions were compared using Fisher’s exact test. Distributions were compared using Mann-Whitney test.

Results

Subjects

In total we analyzed 55 samples (28 CSF samples and 27 plasma samples) from 14 individuals. Baseline characteristics for the study population from their first study visit are shown in Table 1, six subjects were studied at more than one time point.

HIV-1 RNA concentrations in CSF and plasma

Table 1 summarizes the results of SCA HIV-1 RNA concentration measurements while complete results are provided in Table 2. Since, as noted above, sample volume affects the lower limit of detection for SCA, we first compared the volumes for CSF and plasma samples to ensure that a difference in the volumes did not cause a bias in our measurements. There was no significant difference in the volumes (P=0.47). The internal standard failed for three samples, and these results were excluded from analysis. This left measurements from 52 samples (26 CSF and 26 plasma) for analysis. Median and range for the measurements

are shown in Table 1. The proportion of samples with detectable HIV-1 RNA was significantly lower for CSF than plasma (P=0.02). HIV-1 RNA concentrations were also significantly lower in the CSF than in the plasma samples (P<0.0001). Of note, a sample from an individual who had been classified as an EC subject (836) according to the study definition above had a HIV-1 RNA concentration of 189 copies/mL in plasma at the time of sampling for our study, yet interestingly maintained control of infection in the CNS with an undetectable HIV-1 RNA concentration in CSF by SCA (Table 2). For six of the 14 subjects we had samples from multiple time-points. Among the subjects with samples from multiple time points the CSF concentrations varied between <0.3 to 0.6 copies of HIV-1 copies/mL and plasma concentrations between <0.2 to 45 copies/ mL. HIV-1 RNA could be detected in the CSF in 2/6 subjects and in plasma in 4/6 subjects (Table 2). Subject 769 (Table 2) was of special interest since samples were available from primary infection with the first sampling estimated to be at 13 weeks after HIV-1 exposure. HIV-1 RNA concentrations fluctuated in plasma between <0.5 copies/mL and 41 copies/mL while CSF HIV-1 RNA was maintained at very low-levels (<0.3 to 0.6 copies/mL). Plasma measurements using a standard assay (Abbott RealTime HIV-1, Abbot Laboratories, Abbot Park, IL, USA) with a limit of detection of 40 copies/ml showed emergence of detectable HIV-1 RNA at the two latest time points, corresponding with the increase in viral load measured by the SCA.

Discussion

We have previously reported that ECs control CNS HIV-1 infection well [15]. In contrast to the previous report, this study demonstrated, using a more sensitive assay for HIV-1 RNA concentration measurement and analyzing more samples from more subjects, that ECs can occasionally have detectable HIV-1 RNA in their CSF. We found that 5/26 of CSF samples were positive for HIV-1 RNA, as shown in Table 1. The absence of HIV-1 RNA in the CSF of most ECs and the very low-levels detected in the CSF of some ECs could be due to two different reasons: 1) CNS HIV-1 infection occurs in ECs but is very well controlled or 2) HIV-1 does not establish infection in the CNS in ECs but occasionally HIV-1 infected cells migrate into the CSF from the periphery. A previous report has demonstrated that viral evolution in the plasma may occur in ECs with a median HIV-1 RNA concentration of 1 copy/mL [11], so even exceeding low levels of HIV-1 RNA in the CSF may not definitively indicate complete lack of ongoing replication within the CNS.

The HIV-1 RNA concentrations were significantly lower in the CSF than in the plasma of ECs (P<0.0001). HIV-1 RNA was less frequently detected in the CSF compared to the plasma (P=0.02). The fact that we could measure HIV-1 RNA in the plasma of ECs is consistent with prior reports of measurable HIV-1 RNA in the plasma of ECs [17–19]. Our findings that HIV-1 RNA levels in CSF are lower than in plasma are consistent with measurements in these fluids for both untreated and successfully treated individuals [14, 20, 21].

For the 6 ECs where we had samples from multiple time-points we could see fluctuating concentrations of HIV-1 RNA in plasma in 4/6 sample series while concentrations were maintained at a very low level in CSF. This could be an example of viral replication generating immune-escape mutations followed by adaptations in the immune response to

regain control over the infection in plasma while maintaining immune control in CSF (Table 2). One of the ECs (subject 769) was sampled since primary infection. In this case we also noted fluctuating levels of HIV-1 RNA in plasma with very low-levels of HIV-1 RNA in the CSF (Table 2). However, HIV-1 RNA was detectable in CSF and plasma at the two last time-points which could indicate that this is the beginning of a change in the individual's status as an EC. Early viral control in plasma that is lost over time has previously been described by Goujard et al [22]

A weakness of this study is that we did not ensure the primers and probe matched the viral strains in the ECs by sequencing as has been done by an earlier study using SCA to quantify the HIV-1 RNA concentration in plasma from ECs. In this study a primer or probe mismatch that was thought to be significant was found in 11% of the 62 ECs from whom it was possible to obtain a sequence [19]. Our reported numbers could therefore underestimate the prevalence of HIV-1 RNA in CSF and plasma.

In conclusion, using a more sensitive method in a larger study population this study confirms our previous finding that ECs also control CNS HIV-1 infection well. In contrast to our previous findings this study shows that HIV-1 can be detected in the CSF of some ECs although at significantly lower levels than in the plasma. It is however unclear if this means that virions are produced within the CNS or if this is due to migration of infected cells into the CNS. These results may suggest that viral eradication strategies that mimic the natural host mechanisms underlying elite control of infection may also provide effective control of CNS HIV infection, though this will need to be tested directly.

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Table 1

Baseline characteristics and SCA viral load measurements

Number	14
Gender (M/F)	9/5
Age (mean years, range)	51.5 (31–62)
Blood T-Cells (mean cells/uL, range)	
CD4+	736 (429–1738)
CD8+	702 (196–2332)
CSF WBC (mean per uL, range)	2 (0–7)
CSF Protein (mean mg/dL, range)	37.5 (24–62)
Volume (mean mL, range)	
Plasma	6.5 (1–8)[1]
CSF	6.5 (4–8)
Proportion of positive samples	
Plasma	14/26[2]
CSF	5/26
Viral load (mean HIV-1 RNA copies/mL, range)	
Plasma	0.8 (0.1–189)[3]
CSF	0.2 (0.1–6)

[1] Mann-Whitney P=0.47

[2] Fisher's exact test P=0.02

[3] Mann-Whitney P=< 0.0001

Table 2

HIV-1 RNA concentration measurements for all study participants and all time points.

				HIV-1 RNA concentration (HIV-1 RNA copies/mL)	
Subject	**Gender**	**Age (years)**	**Months since first sample**	**Plasma**	**CSF**
514	F	42		<0.2	**0.2**
			6	**3**	<0.3
			12	**11**	<0.3
			18	<0.3	<0.3
566	M	43		**13**	**0.3**
609	M	34		<0.4	<0.3
			73	<0.3	<0.3
652	F	52		**9**	<0.3
690	F	53		<0.6	<0.3
			11	<0.9	<0.2
			25	<0.3	<0.5
708	F	62		<0.4	**0.3**
727	M	37		**0.8**	<0.3
			5	<0.3	<0.3
736	M	51		**0.7**	<0.3
			20	**45**	<0.3
			33	**3**	<0.5*
764	M	55		**26***	**6**
807	M	54			<0.4
825	F	47		<2	<0.4
836	M	54		**189**	<0.3

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				HIV-1 RNA concentration (HIV-1 RNA copies/mL)	
Subject	**Gender**	**Age (years)**	**Months since first sample**	**Plasma**	**CSF**
842	M	57		<0.3	<0.3
769	M	31		**17**	<0.3
			5	**21**	<0.5
			11	<0.5	<0.4
			19	**2**	<0.3
			24	**34**	**0.1**
			30	**40**	**0.6***

Bold figures represent measurement where HIV-1 RNA could be detected. Values marked with * indicate internal standard failure indicating that extraction was suboptimal and actual measurement could be higher. In column for gender “M” is male and “F” is female. The lower-level of detection varied since sample volume varied

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