Clinical Correlates of Human Immunodeficiency Virus–1 (HIV-1) DNA and Inducible HIV-1 RNA Reservoirs in Peripheral Blood in Children With Perinatally Acquired HIV-1 Infection With Sustained Virologic Suppression for at Least 5 Years

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Background. The Early Pediatric Initiation Canada Child Cure Cohort (EPIC⁴) study is a prospective, multicenter, Canadian cohort study investigating human immunodeficiency virus–1 (HIV-1) reservoirs, chronic inflammation, and immune responses in children with perinatally acquired HIV-1 infection. The focus of this report is HIV-1 reservoirs and correlates in the peripheral blood of children who achieved sustained virologic suppression (SVS) for ≥ 5 years.

Methods. HIV-1 reservoirs were determined by measuring HIV-1 DNA in peripheral blood mononuclear cells and inducible cell-free HIV-1 RNA in CD4⁺ T-cells by a prostratin analogue stimulation assay. HIV serology was quantified by signal-to-cutoff ratio (S/CO).

Results. Of 228 enrolled participants, 69 achieved SVS for ≥ 5 years. HIV-1 DNA, inducible cell-free HIV-1 RNA, and S/COs correlated directly with the age of effective combination antiretroviral therapy (cART) initiation ($P < .001$, $P = .036$, and $P < .001$, respectively) and age when SVS was achieved ($P = .002$, $P = .038$, and $P < .001$, respectively) and inversely with the proportion of life spent on effective cART ($P < .001$, $P = .01$, and $P < .001$, respectively) and proportion of life spent with SVS ($P < .001$, $P = .079$, and $P < .001$, respectively). Inducible cell-free HIV-1 RNA correlated with HIV-1 DNA, most particularly in children with SVS, without virologic blips, that was achieved with the first cART regimen initiated prior to 6 months of age ($\rho = 0.74$; $P = .037$) or later ($\rho = 0.87$; $P < .001$). S/COs correlated with HIV-1 DNA ($P = .003$), but less so with inducible cell-free HIV-1 RNA ($P = .09$).

Conclusions. The prostratin analogue stimulation assay, with its lower blood volume requirement, could be a valuable method for evaluating inducible HIV-1 reservoirs in children. Standard commercial HIV serology may be a practical initial indirect measure of reservoir size in the peripheral blood of children with perinatally acquired HIV-1 infection.

Keywords. HIV-1 DNA; inducible RNA; child; reservoir; prostratin analogue.

The initiation of combination antiretroviral therapy (cART) early in human immunodeficiency virus–1 (HIV-1) infection has been associated with lower HIV-1 reservoirs in peripheral blood

in both adults and children [1–9]. Furthermore, posttreatment control, whereby the HIV-1 viral load (VL) remains undetectable for a prolonged period after cessation of cART, has been observed in up to 15% of adults started on cART during early infection, despite unfavorable human leukocyte antigen (HLA) markers [10–12]. While there are no equivalent cohort data for children, several case reports support the possibility of prolonged virologic remission after treatment cessation. In the “Mississippi baby,” cART was started at 30 hours of life, virologic suppression (<48 copies/mL) confirmed at 29 days of life, and treatment stopped at 18 months of life, after which the VL remained undetectable for 21.9 months [13, 14]. In another case from France,

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virologic remission lasting more than 12 years was observed after treatment cessation in a child who earlier had received 5.5 to 6.5 years of cART, beginning at 3 months of age [15].

Measuring the size of the HIV-1 reservoir in a pediatric population is a challenge, because the volume of blood available for sampling is limited. Therefore, HIV-1 DNA, a sensitive and reproducible marker of HIV-1 persistence, is often used [2–9]. However, it is well established that HIV-1 DNA measures largely overestimate the size of the replication-competent HIV-1 reservoir, since the majority of HIV-1 genomes are defective [16, 17]. More recently, the robust correlation and close approximation of reservoir size by inducible cell-free HIV-1 RNA in CD4⁺ T-cells and the quantitative viral outgrowth assay (QVOA) was demonstrated [18]. We used a novel assay, based on prostratin analog (SUW013 [Stanford University Wender lab compound 013]) stimulation of CD4⁺ T-cells, to measure the inducible HIV-1 reservoir in the CD4⁺ T-cells of children with perinatally acquired HIV-1 infections [19].

The main objectives of the present study were to investigate the cross-sectional correlates of HIV-1 reservoir size in the peripheral blood of children with perinatally acquired HIV-1 infection who had a sustained virologic suppression (SVS) of at least 5 years duration, and to evaluate the utility of the prostratin analogue stimulation assay [19] as a measure of the reservoir size in children.

METHODS

Study Design

The Early Pediatric Initiation Canada Child Cure Cohort (EPIC⁴) study is a prospective, multicenter, Canadian cohort study investigating HIV-1 reservoirs, chronic inflammation, and immune responses in children with perinatally acquired HIV-1 infection. Children and young adults were recruited from the 8 major pediatric HIV care centers across the country. The current report is a cross-sectional analysis of predictors of HIV-1 reservoir size in peripheral blood in the subset of children who had achieved SVS for at least 5 years. Children who fulfilled this criterion were categorized *a priori* into 3 groups: Group 1 consisted of those initiated on effective cART during the first 6 months of life and who achieved SVS on their first cART regimen; Group 2 were those initiated on effective cART at or after 6 months of life and who achieved SVS on their first cART regimen; and Group 3 consisted of those who had failed at least 1 cART regimen, but who achieved SVS on a subsequent regimen. The study was approved by the Research Ethics Boards of all participating institutions. Voluntary informed consent was provided by the participant or their legal guardian, as appropriate.

Definitions

SVS was defined as continuous VL measurements below the level of detection (target not detected, <20, <40, and <50 HIV-1 RNA copies/mL plasma, by different assays over time and at

different centers), allowing for virologic blips. Effective cART was defined as cART that led to SVS. A virologic blip was defined as a detectable VL <500 HIV-1 RNA copies/mL of plasma, if the VL measurements immediately preceding and following the blip were below the limit of detection. As previously described, the proportion of life on effective cART refers to the sum of all time periods during which the patient received effective cART associated with SVS, in days (numerator), divided by the patient age, in days (denominator) [20]. The proportion of life with SVS was defined as the sum of all time periods during which SVS was achieved, in days (numerator), divided by the patient age, in days (denominator).

Laboratory Methods

Total HIV-1 DNA was quantified as previously described [21, 22]. Briefly, total peripheral blood mononuclear cells (PBMCs) were digested with proteinase K. Then, a first round of polymerase chain reaction (PCR) preamplification was performed directly on cell lysates using long terminal repeats (LTR)-gag amplification. A nested real-time PCR was then carried out on a Rotor-Gene Q instrument (Qiagen, Mississauga, Canada) using TaqMan probes. The number of copies of the CD3 gene was determined, to accurately quantify the number of cells in each reaction. Results were expressed as HIV-1 DNA copies per 10⁶ PBMCs.

The level of inducible cell-free HIV-1 RNA in CD4⁺ T-cells was measured using the prostratin analogue stimulation assay [19]. Briefly, CD4⁺ T-cells were negatively purified from PBMCs using the EasySep Human CD4⁺ T-cell Isolation Kit (STEMCELL Technologies, Vancouver, Canada) and resuspended (10⁷ cells/mL) in Roswell Park Memorial Institute (RPMI) medium, supplemented with 10% fetal bovine serum, 1000 IU/mL interleukin-2 (IL-2), and 50 nM of the SUW013 prostratin analogue (compound 11c in reference 19) [19]. After 48 h of incubation at 37°C, viral RNA were measured in supernatants using the droplet digital PCR method [23]. Results of the prostratin analogue stimulation assay were expressed as number of copies of HIV-1 RNA produced by 10⁶ CD4⁺ T-cells.

Ultrasensitive VL testing was performed on plasma samples using the droplet digital PCR method. Briefly, HIV-1 RNA was extracted from 1000 µl of a plasma sample, or from 1000 µl of supernatant from prostratin analogue stimulation, with the Nuclisens easyMAG (BioMerieux, Marcy-l'Étoile, France). First-strand cDNA synthesis was performed using SuperScript VILO Master Mix (ThermoFisher Scientific, Waltham, MA). The 1-step droplet digital PCR reaction mixtures were done in triplicates for negative and positive controls, while tested samples were amplified across 6 reactions. Droplets were generated from the PCR reaction mixtures in DG32 Automated Droplet Generator Cartridges, using the Automated Droplet Generator (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. PCR was performed using a C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). The fluorescence

intensities of each droplet from the samples were measured using the QX200 Droplet Reader (Bio-Rad Laboratories, Hercules, CA) and analyzed in the QuantaSoft software.

HIV serology was performed using the Architect HIV-1/2 Ag/Ab Combo Test (Abbott Diagnostics, Mississauga, Canada). The strength of the serologic response was quantified as the signal-to-cutoff value (S/CO) of the assay.

Statistical Analysis

Statistical analysis was performed using SAS statistical software, version 9.4 (SAS Institute Inc., Cary, NC). Demographic characteristics were summarized using proportions for categorical variables and medians with interquartile ranges (IQR) for continuous variables. The comparisons of baseline characteristics between groups were done using the chi-square test, Fisher's exact test, or Wilcoxon rank sum test, as appropriate. For the analyses of reservoir size according to group, the Wilcoxon rank sum test or

Fisher's exact test was performed. Regression analyses (linear or logistic, as appropriate) were also performed to adjust for potential confounders (reservoir sizes on $\log_{10}$ scale for linear regression). Variables were selected for inclusion in the model on the basis of potential clinical relevance or a P value of <0.1 in the univariate analysis. Baseline characteristics that were highly dependent on the group definition were not included, to avoid overadjustment. For the secondary analysis, correlates of reservoir size (HIV-1 DNA, inducible cell free HIV-1) and quantitative HIV-1 serology were evaluated. Correlates of reservoir size (HIV-1 DNA, inducible cell free HIV-1) and quantitative HIV-1 serology were investigated using the partial Spearman's rank correlation coefficient (ρ), while adjusting for potential confounders.

RESULTS

Of 228 children and young adults with perinatally acquired HIV-1 infection enrolled in EPIC⁴, 69 (30.3%) had achieved SVS

Table 1. Baseline Characteristics According to Age of Effective Combination Antiretroviral Therapy Initiation

Parameter	Group 1 (n = 10)	Group 2 (n = 30)	Group 3 (n = 29)	PValue
Current age, in years	10.1 (8.4, 13.2)	15.7 (13.1, 17.6)	17.0 (14.4, 18.1)	<.001
Biological sex, % male	30.0%	50.0%	72.4%	.04
Ethnicity/race	...	...	...	.50
African/Caribbean/Black	55.6%	62.1%	44.8%	...
White	22.2%	17.2%	13.8%	...
Indigenous	11.1%	6.9%	27.6%	...
Other	11.1%	13.7%	13.7%	...
Canadian born, %	100%	40%	72.4%	.001
CDC clinical category, worst ^a	...	...	...	.19
None/mild, N/A	71.5%	58.3%	24.0%	...
Moderate, B	14.3%	16.7%	36.0%	...
AIDS defining conditions, C	14.3%	25.0%	40.0%	...
CDC immunological category, worst ^a	...	...	...	.18
No suppression	57.1%	24.0%	34.6%	...
Moderate suppression	42.9%	48.0%	26.9%	...
Severe suppression	...	28.0%	38.5%	...
Current ART	...	...	...	.80
NNRTI-based cART	30.0%	48.3%	40.7%	...
PI-based cART	50.0%	34.5%	33.3%	...
INSTI-based cART ^b	20.0%	17.2%	25.9%	...
Current CD4 count, cells/ μ L	841 (533, 1145)	670 (510, 839)	640 (510, 773)	.29
Current CD4%	38.0 (36.0, 42.0)	36.5 (32.0, 41.0)	36.0 (32.0, 40.0)	.55
CD4% nadir	24.5 (18.0, 32.0)	21.5 (12.0, 29.0)	19.0 (12.0, 25.0)	.15
Viral load peak, log	5.02 (4.83, 5.70)	4.95 (4.12, 5.63)	5.67 (4.99, 5.90)	.03
Virologic blips	20.0%	56.7%	34.5%	.07
Age at start of effective cART, years	0.2 (0.1, 0.4)	3.2 (1.5, 5.4)	6.8 (3.2, 8.7)	<.001
Age at SVS, years	0.8 (0.4, 1.4)	4.3 (2.2, 6.4)	8.5 (3.7, 9.5)	<.001
Duration of SVS, years	8.7 (7.2, 10.3)	10.5 (7.5, 13.8)	8.0 (6.3, 10.1)	.04
Proportion of life on effective cART	0.98 (0.95, 0.99)	0.82 (0.62, 0.89)	0.65 (0.53, 0.84)	<.001
Proportion of life with SVS	0.94 (0.83, 0.97)	0.74 (0.53, 0.83)	0.57 (0.47, 0.77)	<.001

Group 1 had effective cART initiated at <6 months of life, with SVS on the first cART regimen; Group 2 had effective cART initiated at ≥ 6 months of life with SVS on the initial regimen; Group 3 failed at least 1 cART regimen, but later achieved SVS on another regimen. Results are shown as medians with IQRs for continuous variables and as proportions for categorical variables.

Abbreviations: AIDS, acquired immunodeficiency syndrome; cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SVS, sustained virologic suppression.

^aThe worst CDC clinical category was unknown for 13 cases and the worst immunologic category was unknown for 11 cases.

^bIncludes children on INSTI + NRTI and INSTI + PI + NRTI.

Figure 1. Impact of age of initiation of effective cART on the size of the viral reservoir, measured by quantitative assay of HIV-1 DNA in PBMCs (A), copies of HIV-1 RNA produced by prostratin analogue stimulated CD4⁺ T-cells (B), and quantitative HIV-1-specific serologic response in children with perinatally acquired HIV-1 infection (C). The horizontal lines correspond to the median and IQR. The

for at least 5 years. This subgroup of children had a median current age of 15.5 years (IQR 12.9, 17.6), median age of effective cART initiation of 3.7 years (IQR 1.5, 8.0), and median duration of SVS of 9.1 years (IQR 6.9, 12.5). The baseline characteristics of the participants, according to group, are described in Table 1. The Group 1 individuals were younger, more likely to be female, more likely to be born in Canada, and tended to have less advanced HIV-1 disease, as defined by less severe worst Centers for Disease Control and Prevention (CDC) clinical category, less severe worst CDC immunologic category, and higher CD4% nadir, and to have had fewer virologic blips. As would be expected by the nature of the group definitions, the early treated group was significantly more likely to have achieved SVS at a younger age and exhibited a higher proportion of life on effective cART and higher proportion of life with SVS.

HIV-1 reservoir size, as measured by HIV-1 DNA in PBMC, was significantly lower in Group 1, compared to Groups 2 and 3 (Figure 1A; Table 2). A similar but nonsignificant trend was observed when the size of the inducible HIV-1 reservoir (cell-free HIV-1 RNA in CD4⁺ T-cells) was estimated using the prostratin analogue stimulation assay (Figure 1B; Table 2). A higher proportion of children in Group 1 had no detectable HIV-1 DNA in PBMC (<1 copy per 10^6 PBMC), compared to Group 2 or 3 (70%, 13.3%, and 14.3%, respectively; $P < .001$). In the regression analysis that adjusted for biological sex, CD4% nadir, VL peak, and virologic blips, the significant difference in HIV-1 DNA level between groups was retained ($P = .001$), as was the nonsignificant trend for inducible cell-free HIV-1 RNA level ($P = .17$).

In a separate analysis of the cohort as a whole, those who had no detectable HIV-1 DNA in PBMC were shown to have achieved SVS at a significantly younger age than those with detectable HIV-1 DNA in PBMC (1.4 years [IQR 0.5, 3.5] versus 5.9 years [IQR 3.5, 8.9], respectively; $P < .001$). Inducible cell-free HIV-1 RNA was detected in similar proportions from all 3 groups (80% in Group 1, 86.7% in Group 2, and 86.2% in Group 3), but there was a significant association between the absence of inducible cell-free HIV-1 RNA and younger age at SVS in the full cohort (1.9 years [IQR 1.2, 3.9] with no inducible cell-free HIV-1 RNA versus 5.5 years [IQR 2.8, 8.8] with inducible cell-free HIV-1 RNA; $P = .03$).

The levels of HIV-1 DNA in PBMC, adjusted for biological sex, correlated directly with the age of initiation of effective cART ($\rho = 0.52$; $P < .001$) and age when SVS was achieved ($\rho = 0.44$; $P = .002$) and correlated inversely with

significant and nonsignificant differences between groups were retained in the analysis adjusted for biological sex, CD4% nadir, VL peak, and virologic blips (data not shown). Abbreviations: cART, combination antiretroviral therapy; HIV-1, human immunodeficiency virus-1; IQR, interquartile range; PBMCs, peripheral blood mononuclear cells; VL, viral load.

the proportion of life spent on effective cART ($\rho = -0.51$; $P < .001$) and proportion of life with SVS ($\rho = -0.41$; $P < .001$; Table 3). Similar, though less robust correlations, were noted for inducible cell-free HIV-1 RNA level in peripheral blood CD4+ T-cells. In an analysis restricted to children with no virologic blips ($n = 40$), adjusted for biological sex, significant correlations of similar magnitudes were retained for all 4 variables in relation to HIV-1 DNA in PBMC (age of initiation of effective cART, $\rho = 0.49$ [$P = .003$]; age when SVS was achieved, $\rho = 0.55$ [$P < .001$]; proportion of life spent on effective cART, $\rho = -0.55$ [$P = .001$]; proportion of life with SVS, $\rho = -0.44$ [$P = .006$]); only age when SVS was achieved and proportion of life on effective cART retained significance for inducible cell-free HIV-1 RNA ($\rho = 0.37$ [$P = .021$] and $\rho = -0.41$, [$P = .013$], respectively).

Quantitative HIV-1 serology, as measured by S/CO, was significantly lower for children in Group 1 than for those in Groups 2 or 3 in both the unadjusted and adjusted analyses (Figure 1C; Table 2). Furthermore, all 3 children with negative HIV-1 serology were from Group 1: 2 were initiated on effective cART within 48 hours of birth and 1 at 1.7 months of life. As was the case for HIV-1 DNA in PBMC and inducible cell-free HIV-1 RNA level in CD4+ T-cells, quantitative HIV-1 serology, adjusted for biological sex, correlated directly with the age of initiation of effective cART and the age when SVS was achieved and correlated inversely with the proportion of life spent on effective cART and the proportion of life with SVS (Table 3). In addition, the duration of SVS correlated inversely with quantitative serology. Quantitative HIV-1 serology correlated directly with HIV-1 DNA in PBMC, but less so with inducible cell-free HIV-1 RNA level (Table 3).

A positive correlation was noted between HIV-1 DNA in PBMC and inducible cell-free HIV-1 RNA level in peripheral blood CD4+ T-cells ($\rho = 0.50$; $P < .001$; Figure 2). This correlation was particularly robust for those children in Groups 1 and 2 who had not had any blips subsequent to achieving SVS

($\rho = 0.74$ [$P = .037$] for Group 1, $n = 8$; $\rho = 0.87$ [$P < .001$] for Group 2, $n = 13$). There was no significant correlation between HIV-1 DNA in PBMC and inducible cell-free HIV-1 RNA level in peripheral blood CD4+ T-cells in Group 3 patients ($\rho = 0.34$; $P = .17$).

DISCUSSION

This study shows direct correlation of reservoir sizes in peripheral blood, as measured by both HIV-1 DNA in PBMC and inducible cell-free HIV-1 RNA in CD4+ T-cells, with both the age at initiation of effective cART and the age at which SVS was achieved [4, 6, 8]. In addition, as recently reported from the EPIICAL (Early-treated Perinatally HIV-infected Individuals: Improving Children's Actual Life with Novel Immunotherapeutic Strategies) Consortium, we observed a strong inverse correlation between the size of the viral reservoir measured with both assays and the proportion of life spent on effective cART or proportion of life with SVS [7]. Taken together, these findings indicate that smaller reservoir size in peripheral blood is a function of earlier treatment initiation, as well as higher proportion of life on effective treatment and with virologic suppression. The findings of this study extend those of other pediatric studies demonstrating lower reservoir size in peripheral blood following the early initiation of effective cART [4–9].

Most pediatric studies have relied on measurement of HIV-1 DNA in PBMC to assess reservoir size [4–9]. While this methodology has the advantages of being easier to perform and requiring smaller blood volumes, it cannot distinguish replication-competent virus, as measured by the QVOA, from defective virus and tends to overestimate the size of the replication-competent reservoir by as much as 100-fold [16, 17, 24]. Poor correlation between the QVOA and HIV-1 DNA measurement have been observed in some [16], but not all, studies [25]. In this study, we demonstrate for the first time that the size of the inducible HIV-1 reservoir in the peripheral blood CD4+

Table 2. Reservoir Size According to Age of Effective Combination Antiretroviral Therapy Initiation

Parameter		Group 1 (n = 10)	Group 2 (n = 30)	Group 3 (n = 29)	P Value ^a
HIV-1 DNA ^b	Median (IQR)	10.0 (10.0, 18.0)	117.2 (57.5, 247.5)	77.3 (29.8, 170.1)	<.001
	% undetectable	70.0%	13.3%	14.3%	<.001
Cell-free HIV-1 RNA ^c	Median (IQR)	3.0 (1.2, 12.0)	5.5 (2.4, 21.7)	14.7 (3.4, 63.6)	.17
	% undetectable	20.0%	13.3%	13.8%	.81
Ultrasensitive VL ^d	Median (IQR)	0 (0, 0)	0 (0, 0)	0 (0, 0)	.67
	% negative	90.0%	81.5%	79.2%	.91
Quantitative serology (S/CO)	Median (IQR)	3.4 (0.2, 22.4)	145.9 (12.4, 348.6)	141.8 (45.9, 303.9)	.002
	% negative	30.0%	0%	0%	.002

Abbreviations: HIV-1, human immunodeficiency virus-1; IQR, interquartile range; PBMC, peripheral blood mononuclear cell; S/CO, signal-to-cutoff ratio; VL, viral load

^aThe unadjusted P values are presented. In the regression analysis adjusting for biological sex, CD4% nadir, VL peak, and virologic blips, no significant changes to the results were observed. Minor adjusted P value changes were in the mean log₁₀ HIV-1 DNA ($P = .001$), percent undetectable HIV-1 DNA ($P = .018$), and mean log₁₀ S/CO ($P < .001$).

^bExpressed as copies per 10⁶ PBMC; <10 copies per 10⁶ PBMC are considered undetectable.

^cExpressed as copies of HIV RNA produced per 10⁶ stimulated CD4+ T-cells; <1 copy per 10⁶ CD4+ T-cells is considered undetectable.

^dLevel of detection set at 5 copies/mL.

Table 3. Correlates of Human Immunodeficiency Virus–1 (HIV-1) Reservoir Size in Peripheral Blood and Quantitative HIV-1 Serology

Variable	HIV-1 DNA		Cell-free HIV-1 RNA		HIV-1 Serology	
	Rho	PValue	Rho	PValue	Rho	PValue
Age at cART initiation, years	0.52	<.001	0.26	.036	0.63	<.001
Age at SVS, years	0.44	.002	0.25	.038	0.65	<.001
Duration of SVS, years	–0.12	.35	–0.08	.54	–0.32	.008
Proportion of life on effective cART	–0.51	<.001	–0.32	.01	–0.66	<.001
Proportion of life with SVS	–0.41	<.001	–0.21	.079	–0.68	<.001
HIV serology (S/CO)	0.34	.005	0.21	.089	...	...

The values shown are adjusted for biological sex. All other variables considered for the adjusted analysis—including country of birth (Canada vs elsewhere), CD4 nadir, peak VL, and virologic blips—were highly correlated with other predictor variables depicted in the table and, therefore, not included in the model. In the univariate analysis, “current CD4 count” was significantly associated with HIV-1 serology ($\rho = 0.28$; $P = .022$); in the adjusted analysis this significance was lost ($\rho = 0.21$; $P = .088$).

Abbreviations: cART, combination antiretroviral therapy; HIV-1, human immunodeficiency virus–1; ρ , Spearman partial correlation coefficient; S/CO, signal-to-cutoff ratio; SVS, sustained virologic suppression.

T-cells of children, as measured by the prostratin analogue stimulation assay, correlates with that measured by HIV-1 DNA. Our results further show that this correlation is most robust among those children who were treated very early, achieved SVS, have had no virologic blips, and have very low levels of the virus in their peripheral blood. The strong correlation between the 2 assays in this subgroup may reflect a circumstance where less cumulative viral replication has taken place, thereby limiting both the reservoir size and the accumulation of defective genomes. This suggests that in children treated very early, virologic blips may be associated with sustained, low-level viral replication, with gradual increase in the proportion of defective genomes.

Previous studies have demonstrated that HIV-1 serology can be negative in perinatally infected children with SVS after initiating cART during the first 3–6 months of life [4, 9, 26]. In the present study, we demonstrate that standard, commercial HIV serology, measuring total antibodies to gp41, p24, and

gp120, quantified by S/CO, correlates with HIV-1 reservoir size in peripheral blood. This contrasts with findings in a cohort of 97 children with perinatally acquired HIV-1 infection in Mali, where anti-gp41 antibody activity, quantified by S/CO, did not correlate with HIV-1 DNA level in PBMC, despite a significant correlation with the age of cART initiation [27]. A more recent publication demonstrated significant association of anti-gp160 and anti-gp41 antibody levels with HIV-1 DNA level in PBMCs [28]. In an analysis of 69 children from the EPIICAL cohort, a Western blot band intensity score correlated directly with the time of cART initiation ($P < .001$) and with HIV-1 DNA ($P = .032$) [29]. In our study, antibody level correlated more robustly with HIV-1 DNA level than with inducible virus level. Our data suggest that the Architect HIV-1/2 Ag/Ab Combo Assay could be used as a relatively accessible and inexpensive first-step measure of reservoir size in the peripheral blood of children with perinatally acquired HIV-1 infection.

A limitation of this study was its cross-sectional design, though this was mitigated by the high recruitment rate of subjects with SVS from all participating centers and, hence, the good sample size. The limited volume of blood that could be drawn from younger children precluded the quantification of very low levels of the virus in peripheral blood (<10 copies/ 10^6 PBMC for HIV-1 DNA; <1 copy/ 10^6 CD4+ T-cells for inducible virus) or evaluation of replication-competent virus using the QVOA, which is considered the current gold standard in terms of reservoir measurement. However, the prostratin analogue stimulation assay correlated well with HIV-1 DNA level and could represent a useful and cost-effective tool for assessment of the inducible HIV-1 reservoir in the peripheral blood of children.

In conclusion, we have demonstrated that the size of the HIV-1 reservoir in peripheral blood correlates directly with the age of effective cART initiation and age of SVS and correlates inversely with the proportion of life spent on effective cART and proportion of life with SVS. We have also shown that the size of the inducible HIV-1 reservoir in peripheral blood CD4+ T-cells can be estimated in children using the prostratin analogue

Figure 2. Correlation of HIV-1 reservoir size in PBMC measured using the HIV-1 DNA assay and cell-free HIV-1 RNA in CD4+ T-cells using the prostratin analogue stimulation assay. Abbreviations: HIV-1, human immunodeficiency virus–1; PBMC, peripheral blood mononuclear cell.

stimulation assay and that the results of this assay correlate well with HIV-1 DNA levels, particularly in children who were treated very early, have achieved SVS, and have had no virologic blips. As such, the prostratin analogue stimulation assay, with its lower blood volume requirement, may become a valuable method for estimating replication-competent HIV-1 reservoirs in children. Finally, we demonstrated that quantified HIV-1 serologic responses, using a standard, commercially available HIV-1 serologic assay, may serve as a practical initial indirect measure of reservoir size in the peripheral blood of children with perinatally acquired HIV-1 infection.

Notes

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