Establishment and Replenishment of the Viral Reservoir in Perinatally HIV-1-infected Children Initiating Very Early Antiretroviral Therapy

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Background. Combination antiretroviral therapy (cART) generally suppresses the replication of the human immunodeficiency virus type 1 (HIV-1) but does not cure the infection, because proviruses persist in stable latent reservoirs. It has been proposed that low-level proviral reservoirs might predict longer virologic control after discontinuation of treatment. Our objective was to evaluate the impact of very early initiation of cART and temporary treatment interruption on the size of the latent HIV-1 reservoir in vertically infected children.

Methods. This retrospective study included 23 perinatally HIV-1-infected children who initiated very early treatment within 12 weeks after birth (n = 14), or early treatment between week 12 and 1 year (n = 9). We measured the proviral reservoir (CD4$^+$ T-cell–associated HIV-1 DNA) in blood samples collected beyond the first year of sustained virologic suppression.

Results. There is a strong positive correlation between the time to initiation of cART and the size of the proviral reservoir. Children who initiated cART within the first 12 weeks of life showed a proviral reservoir 6-fold smaller than children initiating cART beyond this time ($P < .01$). Rapid virologic control after initiation of cART also limits the size of the viral reservoir. However, patients who underwent transient treatment interruptions showed a dramatic increase in the size of the viral reservoir after discontinuation.

Conclusions. Initiation of cART during the first 12 weeks of life in perinatally HIV-1-infected children limits the size of the viral reservoir. Treatment interruptions should be undertaken with caution, as they might lead to fast and irreversible replenishment of the viral reservoir.

Keywords. HIV-1; vertical infection; early antiretroviral therapy; viral reservoir.

Perinatal infection by human immunodeficiency virus type 1 (HIV-1) can be diagnosed during the first days of life in newborns infected in utero or 2–6 weeks after delivery in infants infected during delivery. Compared with older children and adults [1], acute infection in babies is characterized by a sustained high-level plasma viral load (pVL) in the initial years of infection [2, 3] and a high risk of rapid progression to AIDS and death [4]. The rapid reduction of peak pVL that is characteristic of acute HIV-1 infection in adults occurs very slowly during the first months of an infant's life. These

age-associated disparities in the viral kinetics of HIV-1 and the clinical outcome of HIV infection are most likely attributable to developmental differences in the neonatal immune system [5, 6].

AIDS-related mortality in children has decreased significantly with the wide availability of combination antiretroviral therapy (cART). Initially, cART was strongly recommended only in infants with HIV-related symptoms [7]. However, during recent years, multiple studies have suggested the benefit of starting early cART in all HIV-1-infected infants [8–11]. Therefore, international guidelines are now recommending initiation of cART in all HIV-1-infected infants aged less than 1 year regardless of clinical and immunological conditions [12, 13].

Some HIV-1-infected infants who initiate cART soon after birth do not display HIV-1-specific antibodies or cellular responses, thus indicating early control of viral replication [14, 15]. Nevertheless, HIV-1 infection quickly establishes latent reservoirs, mainly in resting memory CD4$^+$ T cells. Although the memory T-cell population in peripheral blood is small in newborns [16], only to develop later in childhood [17], recent findings demonstrate the presence of HIV-1-susceptible memory CD4$^+$ T cells in the gut of newborns [18]. The limitations on establishment of reservoirs facilitated by early cART could play a critical role in achieving natural control of viral replication upon discontinuation of cART, which could be defined as "functional cure" [17, 19–22]. On the other hand, viral reservoirs could provide a persistent source of recrudescent viraemia despite temporary remission of HIV-1 infection after withdrawal of treatment [23], as observed in the so-called Mississippi baby [24]. Therefore, in order to design interventions aimed at achieving functional cure in this population, it is important to understand how very early initiation of cART can affect persistence of HIV-1 in older children with successfully suppressed viraemia.

We retrospectively studied a cohort of perinatally HIV-1-infected children who initiated cART within the first year of life. We compared initiation of cART within the first 12 weeks of life with initiation at a later date to assess the potential limitation of establishment of viral reservoirs in the long-term. Our data show that the extent of the latent infection can be limited by administration of optimal cART shortly after birth, which leads to rapid viral suppression. Some of the infants included in this cohort had undergone treatment interruptions. The analysis of the effects of interruptions on the dynamics of viral reservoir indicates that very low reservoir size is not a prognostic marker of long-lasting HIV remission and that reservoir replenishment driven by viral rebound may be fast and irreversible even in these cases.

MATERIALS AND METHODS

Study Participants

The study was based on 139 children with vertically transmitted infection from the Paediatric Spanish AIDS Research Network Cohort (coRISpe). Only samples from those children who initiated cART within the first year of life were included. The analysis was finally based on samples from 23 children who had maintained viral suppression (≤200 copies/mL) for at least 1 year before sampling. When possible, we selected samples that had been collected at multiple time points. Cryopreserved plasma and peripheral blood mononuclear cells (PBMC) and associated clinical data were provided by the Spanish HIV HGM BioBank [25] and by coRISpe [26]. Clinical classification of AIDS-defining events and immunologic categories were based on international guidelines [27]. The ethics committee of Hospital Gregorio Marañón in Madrid approved the study.

Quantification of Proviral HIV-1 DNA

To evaluate the size of the proviral reservoir, CD4$^+$ T cells were purified from PBMC by negative immunomagnetic separation (CD4$^+$ T Cell Isolation Kit; Miltenyi Biotech), and lysed extracts were used to measure cell-associated total HIV-1 DNA by droplet digital polymerase chain reaction (ddPCR) with 5′LTR or Gag primers and probes, depending on the efficiency of detection in each patient. The 2 primer-probe sets have been previously assessed to be comparable in terms of efficiency and sensitivity by ddPCR on a plasmid containing the IIIB reference HIV-1 sequence (standard 2LTR-CCR5 plasmid, kindly provided by M. Stevenson). However, mismatches or deletions in the viral sequences can prevent from efficient amplification in some patient samples. For that reason all samples were measured in parallel using the 2 primer-probe sets to ensure efficient and reliable proviral absolute quantification. The RPP30 cellular gene was quantified in parallel to normalize sample input. All primers and FAM/HEX-ZEN-Iowa BlackFQ dual-labeled double-quenched probes were purchased from Integrated DNA Technologies [28, 29].

Serological Assessment

The Inno-Lia HIV I/II Score assay (Innogenetics, Ghent, Belgium) was run as previously described [30] to evaluate humoral responses in plasma samples (taken simultaneously with stored PBMCs). The results of antibody testing for HIV-1 antigens gp120, gp41, p31, p24, and p17 were analyzed and classified as positive if at least 2 lines had a rating ≥1+, and both were envelope lines or at least 1 was an envelope antigen and the other p24. Other combinations were considered indeterminate.

Ultrasensitive Viral Load Test

Residual low-level viraemia was determined by ultracentrifugation of up to 7.5 mL of plasma at 170 000 i at 4°C for 30 minutes, followed by viral RNA extraction using the m2000sp Abbot device. HIV-1 RNA copies were quantified in the Abbot m2000rt instrument using the Abbott RealTime HIV-1 Assay and laboratory-defined applications software from the instrument. HIV-1 RNA copies in the low range were determined by

means of a calibration curve set between 1000 and 10 copies/mL (five points at 1/3 serial dilutions: 1000, 300, 100, 30, and 10 in triplicate). The quantification method was validated in triplicate with a positive control (prequantified standard HIV from the World Health Organization) in the range of 128–0.5 copies/mL (9 serial 1/2 dilutions). Similarly, the concentration protocol was validated by running direct vs diluted ultracentrifuged pre-quantified plasma samples, with viral load (VL) ranging from 400 to 10 HIV-1 RNA copies/mL.

Statistical Analysis

We compared numerical and categorical variables between groups using the Mann–Whitney test and Fisher exact test, respectively.

A multiple linear regression model was fitted to define the association between HIV-1 DNA and previously selected clinical variables. Selection was performed by means of a Spearman pairwise correlation test to identify those parameters that were significantly correlated with HIV-1 DNA and as independent as possible between them. The final model—$\log_{10}$ (copies HIV-1 DNA/10^6 CD4$^+$ T cells) = (Age at cART initiation) + (Time to virologic control)—was derived using a bootstrapped Akaike information criterion–based stepwise selection method (Supplementary Figure 1).

Longitudinal changes within groups of children who discontinued or did not discontinue cART and the comparison of the mean change between groups (slope coefficient) were evaluated using linear mixed models: $\log_{10}$ (copies HIV-1 DNA/10^6 CD4$^+$ T cells) = intercept + group + timepoint + group*timepoint. A statistically significant interaction term (group*timepoint) identified significantly different slopes between the groups. To perform these longitudinal analyses, timepoints were defined according to the time since the first determination: 0.5–2 years, 2–2.5 years, 2.5–3 years, and more than 3 years. All analysis were performed using the R package [31].

RESULTS

The study population comprised 23 perinatally HIV-1-infected children, who were grouped into 2 categories: those who initiated cART very early (VET, 0–12 weeks, 14 patients) or early (ET, 12–54 weeks, 9 patients). The first cART regimen consisted of a backbone of 2 nucleoside reverse transcriptase inhibitors (mostly lamivudine and zidovudine) plus either a nonnucleoside reverse transcriptase inhibitor (nevirapine) or a protease inhibitor (nelfinavir or lopinavir/ritonavir). Clinical and demographic characteristics are summarized in Table 1. At sampling, all patients responded effectively to cART and had been virologically suppressed (≤200 copies/mL) for more than 1 year (median time on suppression = 4.5 years [interquartile range (IQR):

3.3–6.9]). At sampling (median age = 8.0 years [IQR: 5.1–10.0]), 17 children were on a protease inhibitor–based regimen.

We first explored the potential association between proviral reservoir size and the main clinical parameters. We found a strong positive correlation between total HIV-1 DNA in CD4$^+$ T cells and the age of the children at initiation of cART (P-value <.0001; Figure 1A). Indeed, children treated within the first 12 weeks of life had a smaller reservoir than children treated later (P-value <.01; Figure 1B). A positive correlation was also found between total HIV-1 DNA in CD4$^+$ T cells and the time needed to achieve viral suppression after initiation of cART (P-value = .03; Figure 1C). However, we did not find statistically significant differences when children were classified as rapid controllers (≤1 year) or slow controllers (>1 year) (Figure 1D). A more accurate analysis, in which the main clinical parameters were fitted into a mixed linear model, confirmed that the most significant parameters contributing to proviral reservoir size were age at initiation of cART (P-value = .002) and time between initiation of cART and virologic control (P-value = .04) (Supplementary Figure 1). The resulting model predicts an increment of 0.1 log in the total HIV-1 DNA content of peripheral CD4$^+$ T cells driven by each 1-month delay in the initiation of cART (Figure 2A). Similarly, an equivalent increment would result from each year of suboptimal treatment. Since the samples were not evaluated at the same timepoints, we ruled out the possibility that the total time under virologic control until sampling could affect the total amount of cell-associated HIV-1 DNA (Supplementary Figure 2A and 2B).

Ultrasensitive viral load quantification was used to compare pVL at the time of sampling (Table 1): residual low-level viraemia was detectable in 7 children (30%), 3 (21.4%) in the VET group and 4 (43.4%) in the ET group, with no significant differences between groups, neither in the frequency of detection nor in median levels.

HIV-1-specific antibody testing was performed to assess the children's serostatus. We analyzed the relationship between HIV-1 serostatus and age at initiation of treatment, time to virologic control and proviral reservoir size. As shown in Figure 2B, a higher proportion of VET children presented negative or indeterminate results: seven of the 14 children in the VET group (50%) vs only one of the 9 ET children (11.1%), probably as a result of early and sustained control of viral replication (Table 2 and Supplementary Table 1). However, this trend was not statistically significant (Fisher exact test P-value = .09), and no association was found between serological status and proviral reservoir size (Mann–Whitney test P-value = .20).

The potential role of an extremely small proviral reservoir as a correlate of protection against viral rebound after interruption of treatment remains controversial. Thus, we studied the effect of discontinuation on reservoir size by comparing the longitudinal dynamics of the latent reservoir in three children who

Table 1. Retrospective Study Cohort Characteristics

		Treatment Initiation		
	Total 23 (100%)	Very Early (VET) 0–12 wk 14 (60.9%)	Early (ET) 12–54 wk 9 (39.1%)	P Value
Subject characteristics				
Sex, No. (%)				
Female	15 (65.2)	8 (57.1)	7 (77.8)	.3998
HIV-1 subtype, No. (%)				
B	19 (82.6)	11 (78.6)	8 (88.9)	1.0000
No B	3 (13)	2 (14.3)	1 (11.1)	
Gestational age				
Median [IQR], wk	38 [36–39]	38 [36–39]	39 [38–41]	.1431
Received prophylaxis, No. (%)				
Yes	11 (47.8)	8 (57.1)	3 (33.3)	.4003
Zenith pVL				
Median [IQR], $\log_{10}$ cp/mL	5.8 [5.3–6.2]	5.6 [5.1–6.2]	6 [5.7–6.1]	.2560
Nadir CD4$^+$ T-cell count				
Median [IQR], cells/mm^3	663 [405–1125]	665 [386–1232]	663 [418–1058]	.9749
Median [IQR], %	26 [14–33]	31 [16–36]	15 [14–26]	.1382
Parameters at cART initiation				
Median age [IQR], wk	10 [6–28.4]	7.2 [2.8–9.6]	32.4 [27.3–35.6]	<.0001
CDC category				
N or A	16 (69.6)	13 (92.9)	3 (33.3)	.0091
B	2 (8.7)	0	2 (22.2)	
C	5 (21.7)	1 (7.1)	4 (44.4)	
CD4$^+$ T-cell count				
Median [IQR], cells/mm^3	1785 [1069–3120]	2733 [1455–3733]	1508 [932–1785]	.0507
Median [IQR], %	43 [24–48]	44 [42–50]	24 [16–35]	.0196
Immune category				
1	14 (60.9)	12 (85.7)	2 (22.2)	.0086
2	6 (26.1)	1 (7.1)	5 (55.6)	
3	3 (13)	1 (7.1)	2 (22.2)	
Initial cART regimen, No. (%)				
With protease inhibitors	18 (78.3)	9 (64.3)	9 (100)	.1157
Parameters at virologic control				
Time since cART initiation				
Median [IQR], yr	0.8 [0.5–4.2]	0.5 [0.4–3.8]	1.4 [0.7–5.3]	.2433
Median age [IQR], yr	1.3 [0.5–4.5]	0.6 [0.5–3.9]	2 [1.3–6.3]	.0725
Parameters at sampling				
Median age [IQR], yr	8 [5.1–10]	7.6 [4.3–9.6]	9.1 [6–10.4]	.3610
cART regimen, No. (%)				
with protease inhibitors	17 (73.9)	9 (64.3)	8 (88.9)	.3401
Time on cART				
Median [IQR], yr	7.9 [4.7–9.8]	7.5 [4.2–9.5]	8.6 [5.4–9.9]	.5495
Time under virologic control				
Median [IQR], yr	4.5 [3.3–6.9]	5.2 [2.9–7.4]	4.1 [3.7–4.8]	.8749

discontinued cART and nine children who continued cART. The 3 children who discontinued belonged to the VET group and had a median of 25 HIV-1 copies per million CD4$^+$ T cells (IQR: 15–68). However, this value increased to 199 copies (IQR: 123–778)

after discontinuation despite virologic control for at least one year after reinitiation of cART (Table 3). Even though only a few patients were evaluated, longitudinal analysis revealed a statistically significant increase in the size of the proviral reservoir in this

Table 1 continued.

		Treatment Initiation		
	Total 23 (100%)	Very Early (VET) 0–12 wk 14 (60.9%)	Early (ET) 12–54 wk 9 (39.1%)	*P* Value
Ultrasensitive pVL, No. (%)				
Undetectable, No. (%)	16 (69.6)	11 (78.6)	5 (55.6)	.6244
Median [IQR] of detectable, cp/mL	10 [4.7–12]	10 [10–11]	5 [4–15]	.6600
CD4+ T-cell count				
Median [IQR], cells/mm³	1356 [1046–1668]	1258 [952–1649]	1416 [1179–1680]	.4310
Median [IQR], %	40 [36–44]	41 [36–43]	38 [37–51]	.7283

Virologic control was defined as 2 consecutive pVL determinations of <200 copies/mL after cART initiation. Values are shown as median [IQR] or number (%). The clinical classification of AIDS-defining events and immunologic categories were based on international guidelines. Prophylaxis with zidovudine, lamivudine, and nevirapine immediately followed by cART was considered as initiation of cART. Data were compared between infants initiating cART at less than 12 weeks of age (VET) and infants initiating cART between 12 and 54 weeks of age (ET) using the Mann–Whitney test and Fisher exact test for numerical or categorical variables, respectively.

Abbreviations: cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; ET, early treatment; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; pVL, plasma viral load; VET, very early treatment.
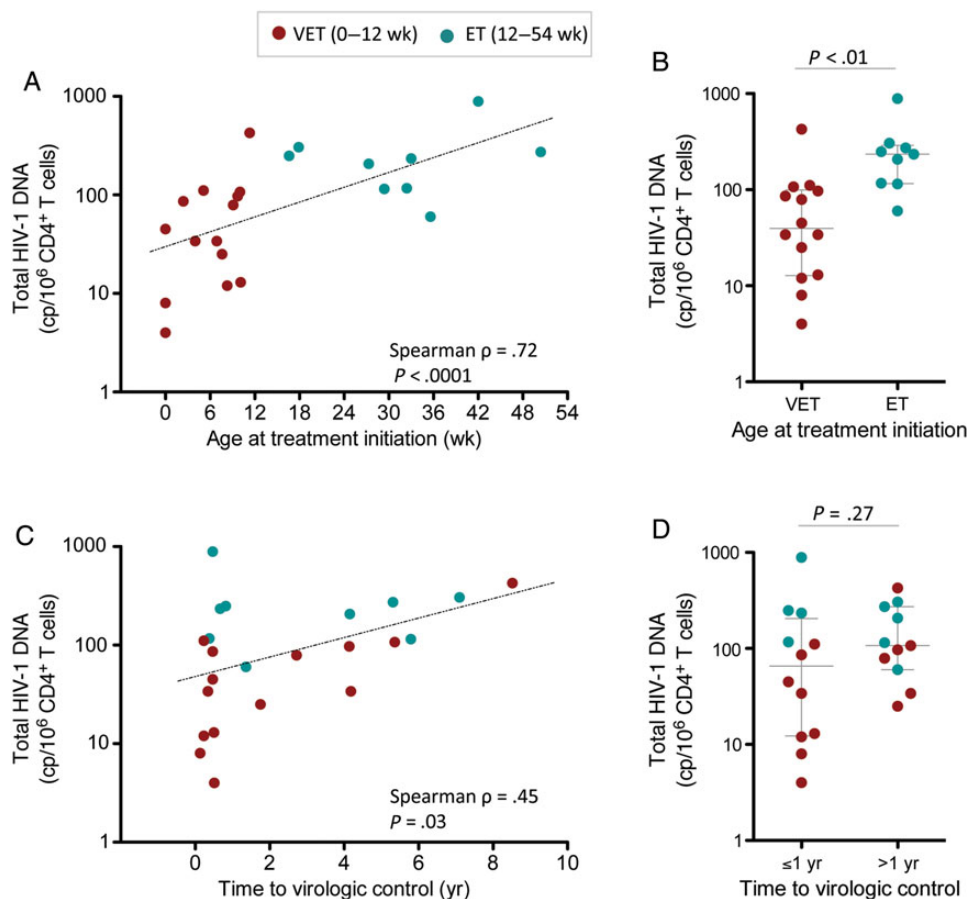
Figure 1. Association between human immunodeficiency virus type 1 (HIV-1) DNA copies/10⁶ CD4+ T cells and age at initiation of treatment (*A* and *B*) or time to virologic control after initiation of treatment (*C* and *D*). Median (interquartile range) levels are shown. The non-parametric Spearman correlation coefficient (ρ) and the associated 2-tailed *P* value (*A* and *C*) and 2-tailed *P* value of grouped comparisons (Mann–Whitney test) (*B* and *D*) are shown. Abbreviations: ET, early treatment; VET, very early treatment.
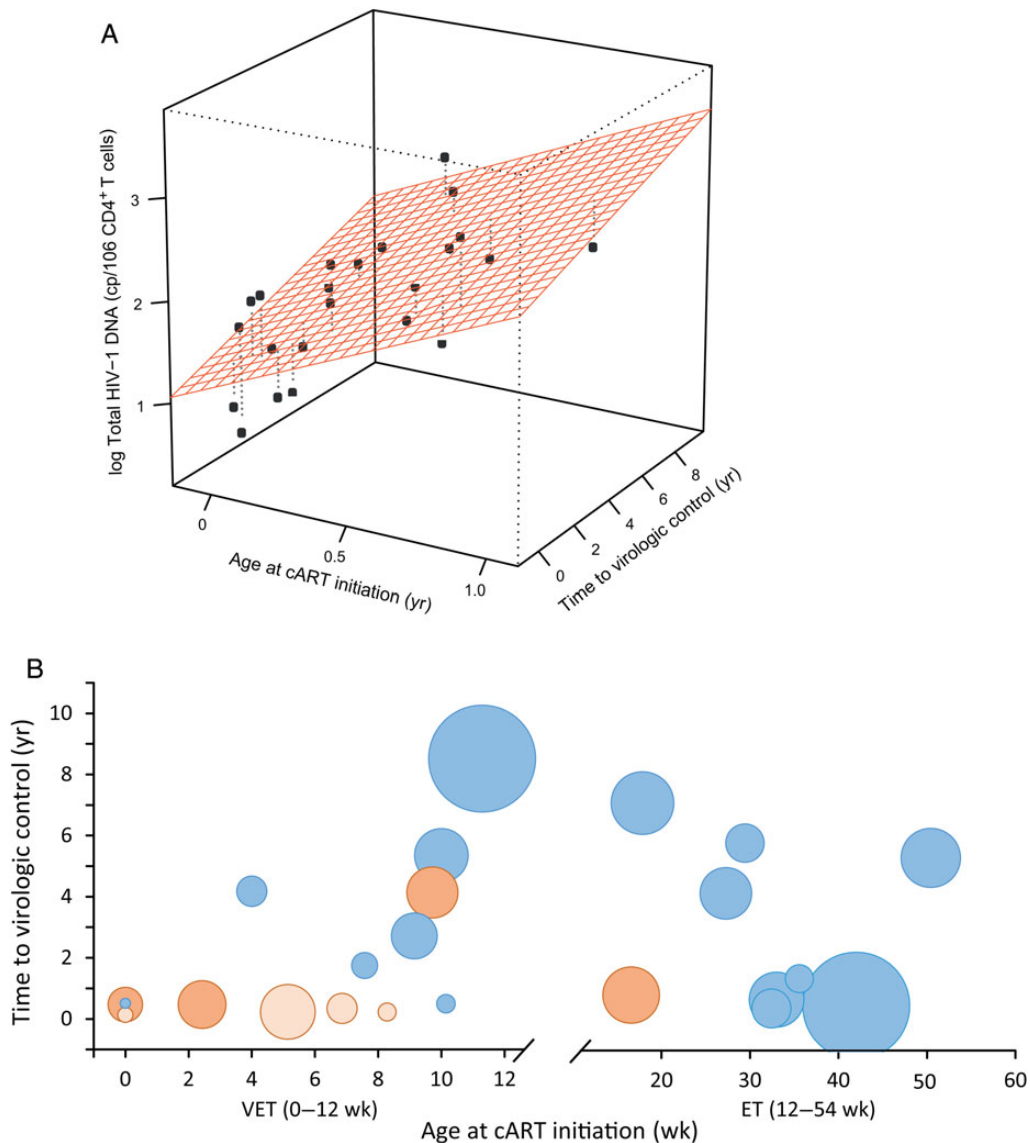
Figure 2. Influence of age at initiation of treatment and time to virologic control after initiation of treatment on proviral reservoir size and humoral profiles. *A*, Three-dimensional graph shows raw data (black circles) with the predicted multiple model (red plane). *B*, Bubble size represents human immunodeficiency virus type 1 (HIV-1) reservoir size (HIV-1 DNA copies/10^6 CD4+ T cells). Measurements in infants who had fully seroconverted are in blue. Patients with negative or indeterminate results in the Inno-Lia assay are indicated in light or dark orange, respectively. Abbreviations: cART, combination antiretroviral therapy; ET, early treatment; VET, very early treatment.

group (slope = 0.29; *P* = .0005), whereas no significant longitudinal changes were found in individuals with complete adherence to cART (slope = −0.02; *P* = .68) (Figure 3*A*). Evaluation of each individual case revealed no correlation between serostatus or initial proviral reservoir size and the extent of reservoir replenishment or time to viral rebound.

Of particular interest is the case of patient (Pt03), whose plasma viraemia levels, CD4+ T-cell counts, and proviral DNA are shown in Figure 3*B*. This patient initiated cART with zidovudine, lamivudine, and nevirapine immediately

after birth and achieved viral suppression 6.5 months later. At 2 years of age, his proviral reservoir size was extremely low (3.8 total HIV-1 DNA copies/10^6 CD4+ T cells), and at 2.2 years of age, treatment was discontinued for 3 weeks (Table 3 and Figure 3*B*). Unexpectedly, this brief treatment interruption led to viral rebound within the first week followed by a rapid increase in pVL up to 500 000 HIV-1 RNA copies/mL. Once cART was restored, pVL decreased rapidly, but the reservoir size increased approximately 50-fold and remained so for the next 3 years.

Table 2. Human Immunodeficiency Virus Type 1 Serostatus

HIV-1 Serostatus, No. (%)	Total 23 (100%)	Treatment Initiation		P Value
		VET (0–12 wk) 14 (60.9%)	ET (12–54 wk) 9 (39.1%)	
Negative	4 (17.4)	4 (28.6)	0	
Indeterminate	4 (17.4)	3 (21.4)	1 (11.1)	.086
Positive	15 (65.2)	7 (50)	8 (88.9)	

The result of the Inno-Lia HIV-1 I/II Score assay is shown as number (%). Data were compared between infants initiating combination antiretroviral therapy (cART) at <12 weeks of age (VET) and infants initiating cART between 12 and 54 weeks of age (ET) using the Fisher exact test.

Abbreviations: ET, early treatment; HIV-1, human immunodeficiency virus type 1; VET, very early treatment.

DISCUSSION

To date, the advantages of initiating cART within the first year in perinatally infected newborns were thought to maximize the potential benefits of limiting a latent reservoir size and enabling reservoir decay, which could probably increase the duration of remission and limit the capacity for reestablishment of viraemia in patients whose treatment is discontinued [20, 32–34]. Our study highlights the importance of very early initiation of cART, if possible within the first 12 weeks of life, and the benefit of optimal virologic control during the first years of life in order to limit the size of the viral reservoir. However, our study also casts doubt on the ability of a small viral reservoir to limit viral rebound after discontinuation (as observed in the case of the Mississippi baby).

In accordance with Persaud and colleagues [35], who highlighted the influence of age at virologic control on peripheral blood HIV-1 reservoir size in perinatally infected adolescents, our results for viral reservoir size in CD4+ T cells also correlate significantly with age at virologic control (P-value <.005; Supplementary Figure 3A), namely, a 4-fold increase in cell-associated DNA in patients who achieved virologic control with more than 1 year of age (Supplementary Figure 3B). However, these results are markedly affected by the fact that 8 out of 9 patients who achieved virologic control during the first year of life started their cART before 12 weeks. We examined our findings in greater depth by independently analyzing age at initiation of cART and time to viral suppression and found that, although both parameters were associated with reservoir size, the effect of the age at initiation of treatment was stronger (Figure 1).

Our results show that the earlier cART is initiated, the smaller the size of the proviral reservoir in peripheral CD4+ T cells in HIV-infected children. This finding is in line with those of a recent study that compared cell-associated HIV-1 DNA in perinatally infected infants initiating treatment before 12 weeks of age with patients initiating treatment during adolescence [36].

Table 3. Characteristics of Patients With Combination Antiretroviral Therapy Discontinuation

	Pt03	Pt08	Pt22
Subject characteristics			
Sex	Male	Male	Female
HIV-1 subtype	B	B	B
Gestational age, wk	38	30	38
Received prophylaxis	No	Yes	No
Zenith pVL, $\log_{10}$ cp/mL	4.0	5.0	6.5
Nadir CD4+ T-cell count			
Cells/mm³	2410	1466	1305
%	36	32	46
Parameters at cART initiation			
Age, wk	0.0	7.6	5.1
CDC category	A	A	A
CD4+ T-cell count			
Cells/mm³	ND	4133	2209
%	48.1	42	47
Immune category	1	1	1
Initial cART regimen	AZT/3TC/NVP	ABC/3TC/LPVr	AZT/ddI/NVP
Parameters at virologic control			
Time since cART initiation, mo	6.1	21	2.8
Age, mo	6.2	22.7	3.9
Parameters of cART discontinuation			
Serostatus before TD	Positive	Positive	Negative
Duration of TD, wk	3	27	284
Highest pVL, $\log_{10}$ cp/mL	5.7	4.4	6.2
Total HIV-1 DNA, cp/10^6 CD4+ T cells			
Before TD	3.8	25.42	111.0
After TD	199.1	46.1	1,356.7
Fold-change	52.4	1.8	12.2

Virologic control was defined as 2 consecutive pVL determinations of less than 200 copies/mL after cART initiation. The clinical classification of AIDS-defining events and immunologic categories were based on international guidelines. Prophylaxis with zidovudine, lamivudine, and nevirapine immediately followed by cART was considered as initiation of cART.

Abbreviations: 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; ddI, didanosine; HIV-1, human immunodeficiency virus type 1; LPVr, ritonavir-boosted lopinavir; ND, not determined; NVP, nevirapine; pVL, plasma viral load; TD, treatment discontinuation.

Moreover, our findings are consistent with those of a study of adult post-treatment controllers who initiated cART during acute HIV-1 infection [19], indicating that early treatment can prevent seeding of long-lived cellular reservoirs. Taken together, our results, which focus on a narrower time frame, clearly show the importance of treating infants immediately after birth to minimize seeding of long-lived cellular reservoirs.

The relevance of testing the HIV reservoir as a potential correlate of viral control after discontinuation has been the subject of intensive research in recent years, ever since the description
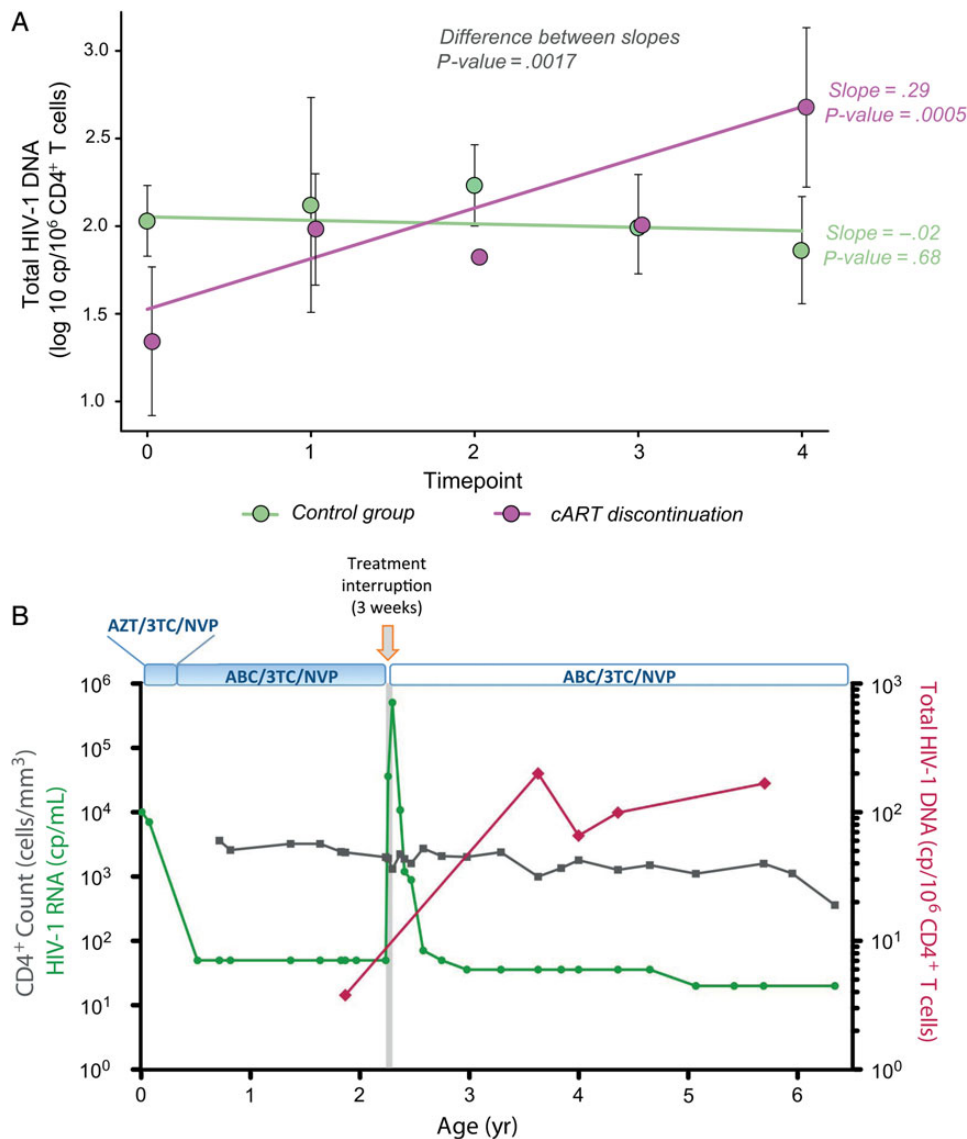
Figure 3. Reservoir size dynamics over time. *A*, Values of total human immunodeficiency virus type 1 (HIV-1) DNA from longitudinal samples are represented as mean ± standard error of the mean from patients who discontinued combination antiretroviral therapy (cART) (pink symbols) or did not discontinue cART (green symbols) after timepoint 0. The slope and *P* values were calculated using the linear mixed model. *B*, Longitudinal follow-up of Pt03. Plasma viral load (green) and CD4+ T-cell count (dark grey) were measured from birth to 6 years of age. Total HIV-1 DNA levels (red) were measured in 4 samples, 1 of which was taken before the 3-week discontinuation of treatment (light grey line). After reinitiation of cART, reservoir size was measured repeatedly over the following 3 years. Abbreviations: 3TC, lamivudine; ABC, abacavir; AZT, zidovudine; NVP, nevirapine.

of the case of the Mississippi baby [24]. Other cohorts of early-treated children with a very low or undetectable proviral reservoir have been established and proposed for treatment interruption programs. Both absence of detectable HIV-1 DNA and absence of low levels of replication-competent viruses in peripheral blood were recently described in a subgroup of HIV-1–infected children who initiated cART within 72 hours of birth, suggesting that early cART could significantly reduce HIV reservoir size [37]. However, definitive proof of functional cure can only be established after long-term virologic control following

discontinuation of cART. Unfortunately, discontinuation in early-treated infected children has invariably led to plasma viral rebound [24, 38, 39]. Indeed, we also showed that despite a small proviral reservoir in the peripheral blood of 3 of the children in our study, plasma viraemia can rebound in only a few days, leading to a marked increase in total HIV-1 DNA in CD4+ T cells (Figure 3A). Although we observed no correlations between serostatus and the time to viral rebound in the children who stopped cART in our study, other immunological parameters, such as cytotoxic T lymphocyte responses or chronic

T-cell activation, could be relevant for remission of HIV and warrant further study.

The origin of viral rebound is unclear. Although it could originate in peripheral blood cells, a large proportion of the viral reservoir is actually located within the gut and other tissues that are not accessed in most studies [40]. On the other hand, replenishment of the reservoir might also become irreversible, as depicted by the sustained level of total HIV-1 DNA in Pt03 during the 3 years after discontinuation (Figure 3B). Although it seems reasonable to think that the reservoir should be as constrained as possible, we cannot conclude that a persistent increase in reservoir size after interruption would necessarily have long-term clinical consequences. Thus, before analytical treatment interruptions could be performed safely in vertically infected children, other reliable biomarkers to predict successful drug-free remission upon discontinuation need to be validated.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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