Influence of Age at Virologic Control on Peripheral Blood Human Immunodeficiency Virus Reservoir Size and Serostatus in Perinatally Infected Adolescents

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**IMPORTANCE** Combination antiretroviral therapy initiated within several weeks of human immunodeficiency virus (HIV) infection in adults limits proviral reservoirs that preclude HIV cure. Biomarkers of restricted proviral reservoirs may aid in the monitoring of HIV remission or cure.

**OBJECTIVES** To quantify peripheral blood proviral reservoir size in perinatally HIV-infected (PHIV+) adolescents and to identify correlates of limited proviral reservoirs.

**DESIGN, SETTING, AND PARTICIPANTS** A cross-sectional study including 144 PHIV+ youths (median age, 14.3 years) enrolled in the United States–based Pediatric HIV/AIDS Cohort Study and receiving durable (median duration, 10.2 years) combination antiretroviral therapy, stratified by age at virologic control.

**MAIN OUTCOMES AND MEASURES** The primary end point was peripheral blood mononuclear cell (PBMC) proviral load after virologic control at different ages. Correlations between proviral load and markers of active HIV production (ie, HIV-specific antibodies, 2–long terminal repeat circles) and markers of immune activation and inflammation were also assessed.

**RESULTS** Proviral reservoir size was markedly reduced in the PHIV+ youth who achieved virologic control before 1 year of age (4.2 [interquartile range, 2.6-8.6] copies per 1 million PBMCs) compared with those who achieved virologic control at 1 to 5 years of age (19.4 [interquartile range, 5.5-99.8] copies per 1 million PBMCs) or after 5 years of age (70.7 [interquartile range, 23.2-209.4] copies per 1 million PBMCs; \( P < .001 \)). A proviral burden of less than 10 copies per 1 million PBMCs in PHIV+ youth was measured in 11 (79%), 20 (40%), and 13 (18%) participants with virologic control before 1 year, at 1 to 5 years, and after 5 years of age, respectively (\( P < .001 \)). Lower proviral load was associated with undetectable 2–long terminal repeat circles (\( P < .001 \)) and HIV-negative or indeterminate serostatus (\( P < .001 \)) but not with concentrations of soluble immune activation markers CD14 and CD163.

**CONCLUSIONS AND RELEVANCE** Early effective combination antiretroviral therapy with prolonged virologic suppression after perinatal HIV infection leads to negligible peripheral blood proviral reservoirs in adolescence and is associated with negative or indeterminate HIV serostatus. These findings highlight the long-term effect of early effective control of HIV replication on biomarkers of HIV persistence in perinatal infection and the utility of HIV serostatus as a biomarker for small proviral reservoir size, although not necessarily for cure.
Despite progress in the prevention of mother-to-child transmission of human immunodeficiency virus (HIV), nearly 240,000 infections occurred among children in 2013. Pediatric HIV infection remains a public health problem, but early treatment improves survival and preserves immune health.

Cure of HIV infection remains elusive owing to the early establishment of viral latency in long-lived resting-memory CD4+ T cells that evade immune surveillance mechanisms and antiretroviral drugs. Sufficiently reducing replication-competent HIV reservoirs to achieve HIV cure or remission so that discontinuation of combination antiretroviral therapy (CART) does not lead to viremic rebound is under intense study. Use of CART during acute HIV infection limits HIV reservoir size and leads to HIV remission in a subset of HIV-infected adults (posttreatment controllers). A recent case report described viral remission in a perinatally HIV-infected (PHIV+) child after 18 months of CART started at 30 hours of age.

In posttreatment controllers, a mean proviral load of 116 copies per 1 million peripheral blood mononuclear cells (PBMCs) before CART discontinuation was associated with virologic control for a median of 6 years after CART discontinuation. Small proviral reservoir size is also reported in adults in whom host immune mechanisms control HIV replication in the absence of CART (elite controllers). Knowing whether long-term virologic suppression with CART can reduce HIV reservoirs durably in perinatal infection is important for studying viral remission or cure. In this study, we examined the effect of age at virologic control after CART on the size of peripheral blood HIV reservoirs in PHIV+ youth with long-term virologic control (≥10 years) and evaluated the association between reservoir size and measures of ongoing HIV replication or production such as 2-long terminal repeat (2-LTR) circles, immune activation, and HIV-specific immune responses.

Methods

Study Participants

The source population for this study was the Pediatric HIV/AIDS Cohort Study (PHACS) Adolescent Master Protocol (AMP), a prospective cohort study designed to evaluate the effect of HIV infection and ART on PHIV+ youth. The PHACS/AMP enrolled 451 PHIV+ children and adolescents and 227 perinatally HIV-exposed uninfected (PHEU) youth receiving care at 15 sites in the United States. The PHACS/AMP was approved by the institutional review board at the Harvard School of Public Health and at each participating site. Written informed consent was obtained from each participant’s parent or legal guardian. Assent was obtained from child participants according to local institutional review board guidelines.

At each study visit, participant demographics, history of ART, HIV viral load (VL), and CD4+ T-cell counts were collected with repository specimens of plasma and cryopreserved PBMCs. Initiation of ART was based on contemporary treatment guidelines. Of 430 PHIV+ children and adolescents enrolled in PHACS/AMP who initiated CART, the 159 who achieved and maintained virologic control as of their last VL measurement before July 2, 2012, were eligible for inclusion (Figure 1). Virologic control was defined as having 2 consecutive VLs of less than 400 copies/mL of plasma after CART initiation and maintaining a VL of less than 400 copies/mL but allowing for isolated VLs of at least 400 copies/mL (ie, intermittent viremia). Within the period of virologic control, the most recent pair (<30 days apart) of plasma and PBMC specimens was analyzed for proviral burden, immune activation and inflammation, and HIV serostatus. If PBMCs were not available, the most recent plasma specimen was used for testing of HIV serostatus and immune activation markers. The final study population included 144 PHIV+ youth with complete information on proviral load, immune activation and inflammation, and HIV serostatus (Figure 1). Ten randomly selected, age-matched PHEU adolescents enrolled in PHACS/AMP served as controls for analysis of immune activation markers.

Quantification of HIV DNA and 2-LTR Circles

We extracted HIV and genomic DNA from frozen PBMC pellets using a commercially available kit (QiAamp DNA Blood Mini Kit; Qiagen) and measured each in triplicate using ultrasensitive droplet digital polymerase chain reaction analysis. Proviral burden was expressed as HIV DNA copies per 1 million PBMCs. Two–long terminal repeat circles were also quantified in the same reaction as used for proviral DNA quantification. With droplet digital polymerase chain reaction analysis, the ribonuclease P gene is used to establish the cellular equivalent of genomic DNA analyzed per reaction.

Determination of Immune Activation

Immune activation was assessed by measuring levels of soluble CD14 (sCD14) and soluble CD163 (sCD163) in stored plasma using commercially available human sCD14 and sCD163 immunoassays (Quantikine Enzyme-Linked Immunoassay kit; R&D Systems). Samples were tested in duplicate. The optical density was determined using a microtiter plate reader (VersaMax Plus ROM, version 1.21; SoftMax). The concentrations of sCD14 and sCD163 were determined by interpolation to a nonlinear standard curve of sCD14 dilutions and sCD163 using commercially available software (GraphPad Prism, version 5; GraphPad Software, Inc). We used an ultrasensitive assay kit (Proinflammatory 9-Plex assay; Meso Scale Discovery) to measure in duplicate the following proinflammatory cytokines: interleukin 1β (IL-1β), IL-2, IL-6, IL-8, IL-10, IL-12p70, granulocyte-macrophage colony-stimulating factor, interferon-γ, and tumor necrosis factor.

HIV-Specific Antibody Detection

Antibody testing for HIV was performed using an enzyme-linked immunosorbent assay (GS HIV-1/2 PLUS O EIA; Biorad) per the manufacturer’s instruction. Western blot testing (Cambridge Biotech HIV-1 Western blot kit; Maxim Biomedical, Inc) assessed antibody responses to gp160, gp120, p66, p55, p51, gp41, p31, p24, and p17 per the manufacturer’s instructions. Results were interpreted as seronegative if no bands were present; as seropositive if any 2 or more of p24, gp41, and gp120/160 bands were present; and as indeterminate if any bands were present but did not meet the criteria for seropositivity.
**Figure 1. Derivation of the Study Population**

- **430 Youths initiated CART**
  - **34** Achieved virologic control\(^a\) before 1 y of age
  - **164** Achieved virologic control\(^a\) from 1 to 5 y of age
  - **204** Achieved virologic control\(^a\) after 5 y of age
  - **28** Did not achieve virologic control\(^a\)
  - **15** Maintained virologic control\(^a\) throughout follow-up
  - **58** Maintained virologic control\(^a\) throughout follow-up
  - **86** Maintained virologic control\(^a\) throughout follow-up
  - **15** With specimens\(^b\)
  - **55** With specimens\(^b\)
  - **85** With specimens\(^b\)
  - **14** With complete information on proviral load, immune activation and inflammation, and HIV persistence
  - **53** With complete information on proviral load, immune activation and inflammation, and HIV persistence
  - **77** With complete information on proviral load, immune activation and inflammation, and HIV persistence

\(^a\) Defined as 2 consecutive viral loads (VLs) of less than 400 copies/mL after CART initiation and maintaining VLs of less than 400 copies/mL (isolated VLs \(\geq 400\) copies/mL between VLs <400 copies/mL, [ie, intermittent viremia] were allowed) to their last VL measurement before July 2, 2012.

\(^b\) Indicates within the period of virologic control, pairs of the most recent plasma and peripheral blood mononuclear cell (PBMC) specimens within 30 days of each other were chosen for laboratory analyses of proviral load, immune activation and inflammation, and HIV persistence, when available. If PBMC specimens were not available, the most recent plasma specimen was chosen for laboratory analyses of immune activation and HIV serostatus.

### Statistical Analysis

The date of the PBMC specimen was considered to be the last visit or end of follow-up for analyses. Demographic and clinical characteristics were compared by age at virologic control after CART initiation (<1 year, 1-5 years, or >5 years) using Fisher exact and Kruskal-Wallis tests as appropriate. Proviral load, 2-LTR circles, and HIV serostatus were also compared by age at virologic control using the Fisher exact and Kruskal-Wallis tests. Distributions of proviral loads among those with and without detectable 2-LTR circles and with positive or negative/indeterminate HIV serostatus were compared using the Kruskal-Wallis test. Values of proviral load below the limit of detection (LOD) were set to the LOD when examining the distribution of proviral load. Immune activation and inflammation markers were compared by age at virologic control and with those of the PHEU group as continuous variables. Their distributions were also compared by detection of 2-LTR circles. We used the Spearman rank correlation coefficient to assess the correlation between each immune activation and inflammation marker and proviral load. All analyses were conducted using SAS statistical software (version 9.2; SAS Institute Inc).

### Results

#### Study Participant Characteristics

The 144 PHIV+ study participants were mostly non-Hispanic black (66.0%), and 48.6% were female (Table 1). The median age at CART initiation was 2.4 months (0.2 years) for the 14 youth achieving virologic control before 1 year of age, 1.9 years for the 53 who achieved control from 1 to 5 years of age, and 5.6 years for the 77 who achieved virologic control after 5 years of age (Table 1). Those who achieved virologic control after 5 years of age were more likely to have received non-CART regimens before CART initiation and took longer to achieve virologic control after CART initiation (Table 1). Higher plasma VL concentrations \((P = \text{.007})\) and CD4\(^+\) T-cell percentage at CART initiation \((P = \text{.07})\) were associated with younger age at virologic control. Younger age at virologic control was also highly correlated with a longer duration of virologic control. The median durations of virologic control from confirmed virologic suppression to the end of follow-up were 11.8, 9.6, and 4.4 years for youth achieving virologic control before 1 year, 1 to 5 years, and after 5 years of age, respectively (Table 1).

At the end of follow-up, the median age of PHIV+ adolescents was 14.3 (interquartile range [IQR], 12.0-16.5) years; adolescents who suppressed HIV after 5 years of age were significantly older than those suppressing at younger ages (Table 1). Reconstitution of CD4\(^+\) T cells occurred to slightly higher percentages in youth with virologic control at younger ages \((P = \text{.03})\), but the increase in median CD4\(^+\) T-cell percentages from CART initiation to the end of follow-up was similar between groups. Among the 10 PHEU adolescents used as a comparison group for the immune activation and inflammation markers, 4 were non-Hispanic black (40%), 3 were female (30%), and their median age at the time of plasma specimen collection was 13.9 (IQR, 12.5-15.4) years.
Table 1. Descriptive Characteristics by Age at Virologic Control

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All PHIV+ Participants (n = 144)</th>
<th>Age at Virologic Control, y&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;1 (n = 14)</td>
</tr>
<tr>
<td>Sex, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>74 (51.4)</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>Female</td>
<td>70 (48.6)</td>
<td>7 (50.0)</td>
</tr>
<tr>
<td>Race/ethnicity, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White non-Hispanic/other</td>
<td>17 (11.8)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Black non-Hispanic</td>
<td>95 (66.0)</td>
<td>10 (71.4)</td>
</tr>
<tr>
<td>Hispanic (regardless of race)</td>
<td>30 (20.8)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>Missing</td>
<td>2 (1.4)</td>
<td>0</td>
</tr>
<tr>
<td>Pre-CART ART regimens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median No. (IQR)</td>
<td>1 (0-2)</td>
<td>0 (0-0)</td>
</tr>
<tr>
<td>Median duration (IQR), y</td>
<td>0.2 (0.0-2.9)</td>
<td>0.0 (0.0-0.0)</td>
</tr>
<tr>
<td>First ever CART type, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI alone</td>
<td>93 (64.6)</td>
<td>10 (71.4)</td>
</tr>
<tr>
<td>PI + NNRTI</td>
<td>19 (13.2)</td>
<td>1 (7.1)</td>
</tr>
<tr>
<td>NNRTI alone</td>
<td>32 (22.2)</td>
<td>3 (21.4)</td>
</tr>
<tr>
<td>Median age at CART initiation (IQR), y</td>
<td>2.7 (0.8-5.8)</td>
<td>0.2 (0.1-0.3)</td>
</tr>
<tr>
<td>HIV VL at CART initiation</td>
<td>Median (IQR), log&lt;sub&gt;10&lt;/sub&gt; copies/mL</td>
<td>4.9 (3.9-5.4)</td>
</tr>
<tr>
<td>No. with missing data</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>CD4 level at CART initiation</td>
<td>Median (IQR), %</td>
<td>27 (20-35)</td>
</tr>
<tr>
<td>No. with missing data</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>CDC class at CART initiation, No. (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N or A</td>
<td>97 (67.4)</td>
<td>12 (85.7)</td>
</tr>
<tr>
<td>B</td>
<td>20 (13.9)</td>
<td>2 (14.3)</td>
</tr>
<tr>
<td>C</td>
<td>27 (18.8)</td>
<td>0</td>
</tr>
<tr>
<td>Median age at virologic control (Q1-Q3), y</td>
<td>6.7 (2.9-10.6)</td>
<td>0.7 (0.6-0.9)</td>
</tr>
<tr>
<td>Median time from CART initiation to virologic control (IQR), y</td>
<td>1.4 (0.4-4.7)</td>
<td>0.5 (0.3-0.7)</td>
</tr>
<tr>
<td>Median age at last visit&lt;sup&gt;e&lt;/sup&gt;</td>
<td>14.3 (12.0-16.5)</td>
<td>12.6 (11.1-14.0)</td>
</tr>
<tr>
<td>HIV VL at last visit&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Median (IQR), log&lt;sub&gt;10&lt;/sub&gt; copies/mL</td>
<td>1.7 (1.6-1.7)</td>
</tr>
<tr>
<td>No. missing</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>CD4 level at last visit&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Median (IQR), %</td>
<td>37 (34-42)</td>
</tr>
<tr>
<td>No. with missing data</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>CD4 count at last visit&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Median (IQR), ratio</td>
<td>1.3 (1.0-1.6)</td>
</tr>
<tr>
<td>No. with missing data</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Median duration of CART use through last visit (IQR), y&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.2 (7.0-12.7)</td>
<td>12.4 (11.0-13.7)</td>
</tr>
<tr>
<td>Median duration of virologic control through last visit (IQR), y&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.2 (4.1-10.5)</td>
<td>11.8 (10.7-13.4)</td>
</tr>
</tbody>
</table>

Abbreviations: ART, antiretroviral therapy; CART, combination ART; CDC, Centers for Disease Control and Prevention; HIV, human immunodeficiency virus; IQR, interquartile range; NA, not applicable; NNRTI, nonnucleoside reverse transcriptase inhibitor; PHIV+, perinatally infected with HIV; PI, protease inhibitor; VL, viral load.

<sup>a</sup> Defined as 2 consecutive VLs of less than 400 copies/mL after CART initiation and maintaining VLs of less than 400 copies/mL (isolated VLs ≥400 copies/mL between VLs <400 copies/mL [ie, intermittent viremia] allowed) to their last VL measurement before July 2, 2012. Percentages have been rounded and may not total 100.

<sup>b</sup> Calculated by the Fisher exact test.

<sup>c</sup> Calculated by the Kruskal-Wallis test.

<sup>d</sup> Classified in Caldwell et al.17

<sup>e</sup> Defined by the date of the peripheral blood mononuclear cell specimen analyzed.
Peripheral Blood HIV Proviral Burden at the Last Visit

The median proviral load in PBMCs at the last visit was significantly lower in participants achieving virologic control before 1 year of age (4.2 [IQR, 2.6-8.6] copies per 1 million PBMCs) compared with those achieving virologic control at 1 to 5 years of age and after 5 years of age, in whom the median proviral loads were 19.4 (IQR, 5.5-99.8) and 70.7 (IQR, 23.2-209.4) copies per 1 million PBMCs, respectively (P < .001) (Figure 2). The droplet digital polymerase chain reaction analysis LOD varied based on the number of analyzed cells available in each sample. The median LOD for HIV DNA was 2.8 (IQR, 1.9-3.8) copies per 1 million PBMCs, and 6 samples had an LOD of 10 copies per 1 million PBMCs, and 2-LTR long terminal repeat repeat.

Ongoing Viremia and Virus Replication During Long-term CART at the Last Visit

Intermittent viremia was rare. Of 3997 plasma VL measurements collected during a median of 7.2 years from the time of virologic suppression to the end of follow-up, only 98 (2.5%) were at least 400 copies/mL and 11 (0.3%) were at least 10 000 copies/mL. Twenty-nine of the 144 adolescents (20.1%) had 2-LTR circles detected in PBMCs. None of the 14 children achieving virologic control before 1 year of age had detectable 2-LTR circles compared with 8 (15%) and 21 (27%) of those achieving control at 1 and 5 years of age or after 5 years of age, respectively (P = .03). No association between 2-LTR circles and intermittent viremia was observed; 27 of the 29 adolescents (93%) with 2-LTR circles did not have intermittent viremia compared with 107 of the 115 adolescents (93%) with undetectable 2-LTR circles (P > .99). Furthermore, none of the 29 participants with 2-LTR circles had HIV-negative or indeterminate serostatus compared with 20.9% of the 115 with undetectable levels of 2-LTR circles (P = .004). The median proviral burden was significantly higher in the 29 adolescents with detectable 2-LTR circles (190.8 [IQR, 78.2-257.6] copies per 1 million PBMCs) compared with 23.2 (IQR, 5.1-75.9) copies per 1 million PBMCs in the 115 adolescents without detectable 2-LTR circles (P < .001).

Immune Activation and Long-term Virologic Control

Among the 9 proinflammatory cytokines analyzed, plasma concentrations of tumor necrosis factor, granulocyte-macrophage colony-stimulating factor, IL-1β, IL-2, and IL-8 were substantially higher in PHIV+ than in PHEU youth (Table 2). Only plasma IL-2 levels differed across the 3 PHIV+ groups, with levels higher in youth with HIV suppression at younger ages. Concentrations of sCD14, a predictor of HIV disease progression and mortality in adults,18,19 did not differ by age at virologic control but were significantly higher in PHIV+ than in PHEU adolescents (median, 1.8 vs 1.3 μg/mL; P < .001) (Table 2). Soluble CD163 concentrations were not significantly higher in PHIV+ than in PHEU youth (484.6 vs 376.3 ng/mL; P = .17) (Table 2). Plasma markers of immune activation did not correlate with proviral DNA level or 2-LTR circle detection.

HIV Serologic Profiles in Perinatal HIV Infection With Long-term CART

Five of the 14 children (36%) achieving control before 1 year of age were HIV seronegative by enzyme-linked immunosorbent assay at their most recent visit compared with 2 of 53 (4%) who achieved virologic control at 1 to 5 years of age and none of the 77 with virologic control after 5 years of age. Younger age at virologic control was also associated with an indeterminate or negative HIV serostatus by Western blot test results compared with 10 of the 53 (19%) achieving virologic control at 1 to 5 years of age and 2 of the 77 (3%) achieving virologic control after 5 years of age (P < .001). Human immunodeficiency virus serostatus was associated with proviral reservoir size. The median proviral load for 24 adolescents with indeterminate or negative HIV serostatus by Western blot analysis was 4.3 (IQR, 3.0-18.3) copies per 1 million PBMCs, which was significantly smaller than 56.7 (IQR, 10.8-150.1) copies per 1 million PBMCs in 120 adolescents with a positive HIV serostatus by Western blot analysis (P < .001) (Figure 3).

Discussion

Although current CART regimens are effective in controlling HIV replication to clinically undetectable levels for years, ex-
Table 2: Distribution of Immune Activation and Inflammation Markers by Age at Virologic Control Among PHIV+ Participants and PHEU Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All PHIV+ Participants (n = 144)</th>
<th>Age at Virologic Control, y</th>
<th>P Value for PHIV+ Cohorts*</th>
<th>PHEU Controls (n = 10)</th>
<th>P Value for PHIV+ vs PHEU Cohorts*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble CD14 level, median (IQR), pg/mL</td>
<td>1.8 (1.5-2.1)</td>
<td>1.9 (1.6-2.3)</td>
<td>1.8 (1.5-2.0)</td>
<td>1.8 (1.5-2.1)</td>
<td>.41</td>
</tr>
<tr>
<td>CD163 level, median (IQR), ng/mL</td>
<td>21.9 (15.7-28.7)</td>
<td>19.6 (17.2-25.7)</td>
<td>21.9 (15.2-26.7)</td>
<td>22.9 (16.3-29.3)</td>
<td>.56</td>
</tr>
<tr>
<td>TNF level, median (IQR), pg/mL</td>
<td>3.7 (1.1-7.9)</td>
<td>3.3 (1.1-6.9)</td>
<td>3.3 (1.8-9.2)</td>
<td>3.7 (1.8-9.2)</td>
<td>.17</td>
</tr>
<tr>
<td>IL-1β level, median (IQR), pg/mL</td>
<td>1.9 (1.5-2.4)</td>
<td>2.4 (2.0-2.4)</td>
<td>1.9 (1.5-2.4)</td>
<td>1.9 (1.5-2.4)</td>
<td>.04</td>
</tr>
<tr>
<td>CD163 level, median (IQR), pg/mL</td>
<td>2.5 (1.3-4.8)</td>
<td>2.5 (2.1-3.0)</td>
<td>2.3 (1.2-4.0)</td>
<td>3.0 (1.5-5.2)</td>
<td>.38</td>
</tr>
<tr>
<td>IL-8 level, median (IQR), ng/mL</td>
<td>18.5 (11.6-26.5)</td>
<td>18.4 (13.2-29.2)</td>
<td>18.6 (9.6-27.9)</td>
<td>18.2 (12.2-23.4)</td>
<td>.88</td>
</tr>
<tr>
<td>IL-10 level, median (IQR), pg/mL</td>
<td>6.4 (4.0-11.1)</td>
<td>6.8 (5.6-7.9)</td>
<td>5.4 (4.0-10.9)</td>
<td>7.0 (3.5-13.5)</td>
<td>.84</td>
</tr>
<tr>
<td>IL-12 level, median (IQR), pg/mL</td>
<td>3.3 (2.7-5.8)</td>
<td>2.9 (2.0-5.9)</td>
<td>3.3 (2.7-5.8)</td>
<td>3.9 (2.7-5.8)</td>
<td>.24</td>
</tr>
</tbody>
</table>

Abbreviations: GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, immune interferon; IL, interleukin; IQR, interquartile range; PHEU, perinatally exposed to human immunodeficiency virus (HIV) but uninfected; PHIV+, perinatally infected with HIV; TNF, tumor necrosis factor. *Calculated by the Kruskal-Wallis test.

existing strategies do not eradicate replication-competent HIV reservoirs, including in PHIV+ children. However, very early CART in these children may limit virus spread into HIV reservoirs and promote HIV remission or cure whereby imminent viremic rebound does not recur when CART is stopped, as was demonstrated in the case of an infant in Mississippi. Approaches to virus eradication in persons with chronic HIV infection in whom reservoirs are established are likely to be most effective in those who achieve marked restriction in proviral reservoir size during CART. Defining the virologic and immunologic end points after standard effective CART for perinatal infection and identifying factors and biomarkers associated with limited proviral reservoir size are critical to identify study participants for proof-of-concept studies aimed at curing HIV infection.

To our knowledge, this cohort-based study is the first to show that younger age at virologic control with standard CART is associated with marked reduction in proviral reservoirs in PHIV+ children surviving to adolescence. Younger age at virologic control gave rise to a unique biomarker profile of small or undetectable peripheral blood HIV proviral load, absent 2-LTR circles, and indeterminate or negative HIV serostatus, suggesting excellent long-term control of HIV replication. The proviral reservoir size was also smaller when virologic control occurred at 1 to 5 years of age compared with after 5 years of age, emphasizing the effect of earlier treatment on proviral reservoir size in perinatal infection. This difference in viral reservoir size as a function of age at virologic control fits with the developmental aspects of memory CD4+ T-cell formation during childhood, whereby memory CD4+ T cells develop slowly over time. Infants are born with few circulating memory CD4+ T-cell...

Figure 3: Distribution of Proviral Load by Human Immunodeficiency Virus (HIV) Serostatus at Last Visit

The limit of detection (LOD) for proviral load varies based on the number of cells available for analysis. Values of proviral load below the LOD were set to the LOD. PBMCs indicates peripheral blood mononuclear cells.

* Indicates comparison between Western blot serostatus by Kruskal-Wallis test.
compartments (<10%) compared with levels in adults (50%). Furthermore, long-lived central memory CD4+ T cells, the predominant reservoir for HIV, are limited in infants, exemplifying the interaction between early virologic control and eventual reservoir size. This finding is consistent with the strong correlation of time to first undetectable VL with the size of the resting CD4+ T-cell latent HIV reservoir at 2 years of age in perinatal infection. However, undetectable levels of HIV provirus in peripheral blood likely still allow for the presence of replication-competent HIV that can rekindle viremic rebound if CART is stopped. The limitation in the current use of undetectable proviral DNA to assess HIV clearance and cure is highlighted by the 2 patients in Boston, Massachusetts, who achieved undetectable proviral DNA levels after bone marrow transplantation; proviral DNA levels rebounded after CART was discontinued. The encouraging single case of drug-free remission in the Mississippi infant prompts the need for a more robust analysis of a larger cohort treated shortly after birth.

Proviral DNA levels are thought to be fairly stable after the first year of CART despite ongoing treatment. Replication-competent HIV reservoirs also show minimal decay during long-term CART. However, more recent studies in adults and children suggest that some decay in HIV proviral DNA levels continues during 10 to 15 years of long-term follow-up. In a PHIV+ youth who initiated CART at a median age of 2 months, decay of proviral load continued over a median of 14 years of virologic suppression, reaching an extraordinarily low level (median, 0.86 log10 copies per 1 million PBMCs). This level is substantially lower than the median proviral load of 2.15 log10 copies per 1 million PBMCs reported in adults initiating CART during acute HIV infection followed by a median of 10 years of virologic suppression, although different techniques were used to measure HIV proviral burden. In the present cross-sectional study, we were unable to assess the independent effects of duration of virologic control and age at virologic control on proviral load, which were highly correlated. Ongoing proviral decay during long-term CART in PHIV+ individuals thus warrants further study. Defining the interaction between age at virologic control, duration of suppression, and decay of peripheral blood HIV reservoirs during long-term CART in perinatal infection is a critical next step for refining therapeutic strategies aimed at achieving HIV remission or cure for this population.

Immune activation as a consequence of HIV infection is a known contributor to HIV-related morbidity and mortality. Treatment during acute HIV infection in adults decreases, but does not reverse, abnormal immune activation. Levels of sCD14, a biomarker of monocyte activation, result from microbial translocation through the intestinal mucosa during HIV infection. Microbial translocation is also increased in early infancy in perinatal HIV infection. In our study, levels of sCD14 remained abnormally high despite long-term virologic control, even in the children who achieved virologic control before 1 year of age with small proviral reservoirs. Studies of the immunopathogenesis of chronic immune activation in patients with an extremely small proviral burden, as reported herein, should provide additional insights into this process and suggest that the initial gut-associated abnormalities induced by HIV infection are not restored with long-term virologic control.

The contribution of ongoing virus replication to the maintenance of proviral reservoirs during CART is controversial. Despite prolonged virologic control (median follow-up, 7.2 years), 2-LTR circles were detected in 20.1% of the study participants and were associated with more than 8-fold larger proviral reservoirs (190.8 vs 23.2 copies per 1 million PBMCs), suggesting ongoing replenishment of HIV reservoirs in a subset of patients despite seemingly effective virologic control during CART. None of the study participants who achieved virologic control before 1 year of age had detectable levels of 2-LTR circles.

Early effective control of HIV replication has been associated with incomplete development of HIV-specific immune responses. The phenomenon of absent HIV-specific antibody responses in PHIV+ children treated with early, effective CART has been reported in as many as 94% of such children who underwent testing at a median age of 21 months and from 47% to 66% of those who underwent testing from 5 to 6 years of age. Seventy-five percent of PHIV+ children with effective virologic control from infancy for a median of 5.5 years had negative or indeterminate test results for HIV but still harbored replication-competent HIV in the resting memory CD4+ T-cell reservoir. The lack of HIV-specific antibodies in this setting likely reflects early control of virus replication with subsequent limited exposure to HIV antigens. We found significantly smaller proviral load among youth who had negative or indeterminate HIV serostatus. Negative or indeterminate HIV serostatus may therefore provide a useful biomarker for small proviral reservoirs in perinatal infection.

Limitations of this study include the lack of analyses of replication-competent proviral reservoirs that require large blood volumes and freshly collected blood samples in addition to longitudinal data to assess the contribution of ongoing proviral reservoir decay to low proviral burden fully during long-term CART for perinatal HIV infection. Analyses of genetic factors that may contribute to long-term virologic control or small reservoir size, such as HLA alleles or heterozygosity of the CCR5 Δ32 mutation, were not performed. Nevertheless, this study is the first, to our knowledge, to comprehensively analyze the virologic and immunologic effects of long-term CART on biomarkers of HIV persistence in a large number of PHIV+ youth, with the identification of unique patterns of persistence based on age at virologic control.

Conclusions

The World Health Organization recommends CART in all HIV-infected children younger than 5 years. Initiating ART within the first few days of life in HIV-infected neonates, as in the infant from Mississippi, to achieve virologic remission or cure in perinatal infection is currently challenging owing to obstacles in early diagnosis in infants and ART drug options for this age group. With CART, PHIV+ children have the potential to survive to adulthood. This study adds evidence that
early effective control of virus replication in perinatal infection with CART, along with prolonged virologic suppression, leads to substantially restricted HIV peripheral blood proviral reservoirs and makes these children and adolescents potential candidates for interventions examining HIV remission or cure.

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