Perinatal CMV Co-Infection Influences the HIV Reservoir in Individuals with Perinatally Acquired HIV

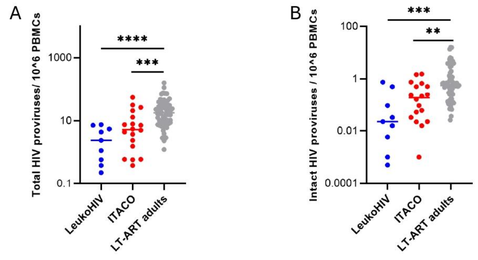
P-02

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BACKGROUND

Early initiation of antiretroviral therapy (ART) limits the size and diversity of the HIV reservoir. In two cohorts of young adults with perinatally acquired HIV (PaHIV) under long-term ART (LeukoHIV and ITACO), we observed a reduced total and intact HIV reservoir (**Fig. 1A** and **B**) compared with adults with horizontally acquired HIV. We hypothesized that the perinatal Cytomegalovirus (CMV) co-infection could have an impact on proviral reservoir composition.

Figure 1



METHODS

We identified 17 PaHIV participants from ITACO and LeukoHIV cohorts based on the availability of complete clinical data, including CMV serostatus and documented timing of CMV infection, as well as comprehensive virological analyses. All participants-initiated ART at a median age of 0.58 years and maintained virologic suppression for a median of 21.2 years. They were stratified into three groups: perinatal, postnatal, or CMV-negative. No significant differences were observed in other clinical characteristics between groups (**Table 1**). Proviral composition analyzed on PBMCs using near-full-length single-genome sequencing (FLIP-seq) was compared across groups.

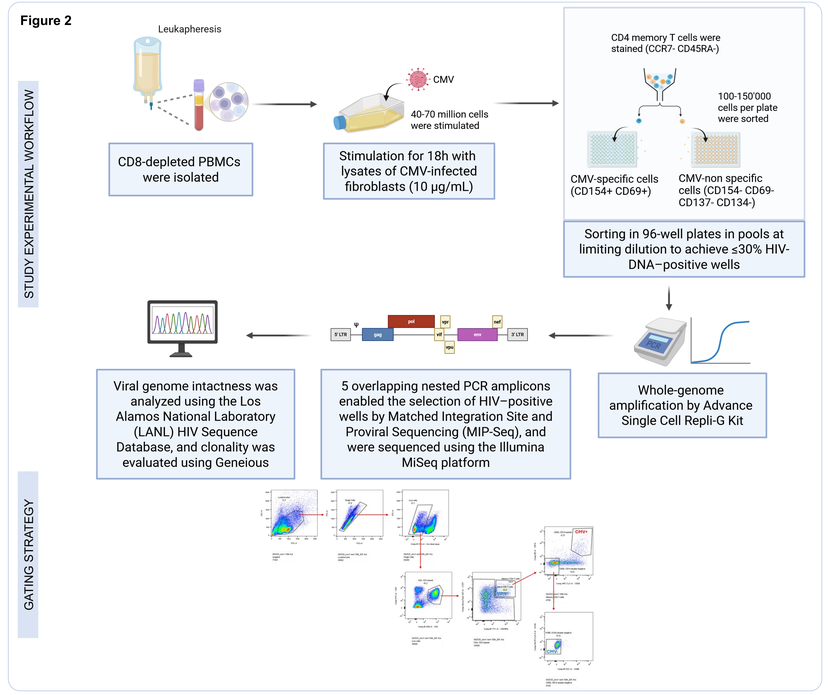
Table 1

Study ID	Gender	Ethnicity	Age at enrollment (years)	Time under suppressive ART (years)	Age at ART start (years)	Lymphocytes absolute count (cell/µl)	CD4 T cells absolute count (cell/µl)	CD8 T cells absolute count (cell/µl)	CD4/CD8 ratio	CMV co-infection
001_009	Female	Hispanic	23.51	18.77	4.74	1492.48	544.76	489.53	1.11	negative
001_016	Female	Caucasian	22.93	22.40	0.54	1502.82	727.36	420.79	1.73	negative
001_026	Female	Caucasian	26.02	25.75	0.27	2679.15	1296.71	723.37	1.79	negative
001_030	Female	Caucasian	14.75	14.75	0.00	1455.51	689.91	301.29	2.29	negative
001_040	Female	Caucasian	28.58	27.66	0.92	977.12	397.69	278.48	1.43	negative
001_042	Female	Black	12.28	11.91	0.37	1520.12	532.04	386.11	1.38	negative
001_007	Male	Caucasian	23.03	22.53	0.50	3221.76	1681.76	844.10	1.99	postnatal CMV
001_011	Male	Asian	19.81	15.19	4.62	2481.36	962.77	622.82	1.55	postnatal CMV
001_014	Male	Black	17.14	16.18	0.96	2465.06	1262.11	576.82	2.19	postnatal CMV
001_024	Male	Caucasian	22.85	18.44	4.42	1821.18	375.16	560.92	0.67	postnatal CMV
001_028	Male	Black	17.78	16.14	1.64	1818.9	603.87	471.10	1.28	postnatal CMV
001_029	Male	Black	24.91	24.30	0.61	3091.66	1082.08	983.15	1.10	postnatal CMV
001_032	Male	Caucasian	17.80	NA	NA	1222.04	409.38	311.62	1.31	postnatal CMV
001_001	Female	Black	22.43	22.12	0.31	1606.22	591.09	390.31	1.51	perinatal CMV
001_022	Female	Caucasian	14.03	13.59	0.44	2187.36	802.76	568.71	1.41	perinatal CMV
001_036	Male	Black	21.23	20.68	0.55	3061.9	1463.59	936.94	1.56	perinatal CMV
001_038	Female	Black	18.62	16.95	1.67	1437.8	375.27	437.09	0.86	perinatal CMV

To characterize the HIV-1 reservoir within CMV-specific and non-specific CD4⁺ T cells in perinatally CMV–HIV co-infected individuals (pCHcoI), we applied an adapted sorting protocol (**Fig. 2**). Given the large number of cells required to detect CMV-specific responses, leukapheresis samples from the LeukoHIV cohort were used. CD8-depleted PBMCs were stimulated, and memory CD4⁺ T cells were bulk-sorted into CMV-specific and CMV-nonspecific populations. Reservoir composition was then assessed.

Co-exposure to CMV during early HIV infection is associated with a larger intact and clonally intact reservoir in PBMCs and may promote the integration of non-clonal intact proviruses into CMV-specific cells.

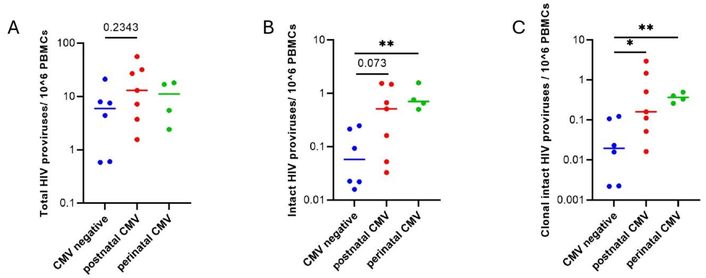
Figure 2



RESULTS

Within PaHIV, individuals with a history of perinatal CMV co-infection showed a higher frequency of intact and clonally intact proviruses (**Fig. 3B** and **C**) compared with those with no CMV exposure. No differences were observed in total reservoir (**Fig. 3A**).

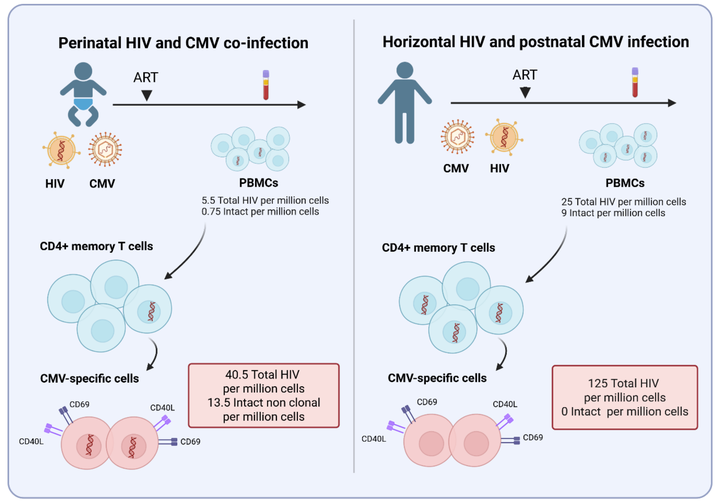
Figure 3



Summary: In youths living with co-infection of HIV and CMV since birth, CMV may influence how HIV hides and persists in certain immune cells early in life.

Due to the extremely low HIV reservoir observed in the LeukoHIV cohort, the full protocol was completed in a single participant with pCHcoI (20.8-year-old female). Reservoir composition was compared with an adult control with horizontally acquired HIV and postnatal CMV infection (**Fig. 4**). In PBMCs, the pCHcoI participant exhibited a markedly lower HIV reservoir than the control, both in total (5.5 vs 25 copies/10⁶ cells) and intact proviruses (0.75 vs 9 copies/10⁶ cells). Within memory CD4⁺ T cells, total HIV reservoir levels did not differ between CMV-specific and CMV-nonspecific populations in either individual. However, qualitative analysis of the pCHcoI participant revealed an enrichment of non-clonal intact proviruses in CMV-specific cells compared with CMV-nonspecific cells (13.5 vs 6.7 copies/10⁶ cells). In contrast, no intact proviruses were detected in CMV-specific cells of the postnatal CMV control; all intact proviruses identified in this individual were clonal and restricted to CMV-nonspecific cells.

Figure 4



CONCLUSIONS

Perinatal CMV co-infection is associated with increased intact and clonal intact reservoir in PBMCs in individuals with PaHIV. Although based on a single pCHcoI participant, our findings suggest that perinatal CMV co-infection may favor the persistence of nonclonal intact proviruses within CMV-specific CD4⁺ T cells. As these cells account 2.5-4% of total CD4⁺ T cells, this enrichment is unlikely to be explained by cell frequency. Overall, these data provide preliminary evidence for a role of perinatal CMV infection in influencing the qualitative features of the early HIV reservoir composition.

ADDITIONAL KEY INFORMATION

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AFFILIATIONS & ACKNOWLEDGE

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