T-Cell Senescence and CMV Positivity and Viremia Status in Perinatally HIV-Infected Children

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ABSTRACT

Background: We examined effects of cytomegalovirus (CMV) co-infection and viremia on CD4+ and CD8+ T-cell activation and senescence over time in a cohort of treated perinatally HIV-Infected (PHIV+) children with severe disease.

Methodology: Pediatric AIDS Clinical Trials Group (PACTG) protocol 366 (P366) was an open-label, multicenter 96 week study where PHIV+ children received 2-4 new drugs (Table 1) depending on their antiretroviral therapy (ART) experience. The analysis included 107 P366 enrollees ≥1 year old with available immune activation data and baseline stored plasma samples. CMV IgG (Genway Biotech Inc.) and CMV DNA (Abbott Molecular) testing were performed on stored plasma specimens. Immunophenotyping of CD4+ and CD8+ T-cells was done at study weeks 0 (baseline), 12, 20 and 40 using flow cytometry in laboratories that participated in the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS (DAIDS) Quality Assurance Program. Immune markers assessing for naïve (CD62L+CD45RA+; CD95-CD28+), activated (CD38+HLA-DR+), apoptotic (CD95+), terminally differentiated (62L-RA+) and senescent (CD28+CD95+) CD4+ and CD8+ T cells were evaluated. Linear mixed models were used to assess associations between baseline CMV status and percentages of 24 phenotypes of CD4+ and CD8+ T-cells at weeks 0, 12, 20, and 40, adjusting for potential covariates. Interaction terms between CMV status and week were added to the models. (Table 1)

Results: The average participant age was 7 years, 58% were Black Non-Hispanic, 55% males; at entry, 98% were on ART; 37% in CDC category C. Forty-one percent were VL responders (Table 1) at week 16 of the new regimen; 19% partial responders; 26% non-responders. Fourteen percent had detectable CMV DNA ("CMV+ viremic"); 49% were IgG positive or had a positive baseline CMV blood or urine test result but no detectable CMV DNA ("CMV+ non-viremic"); 37% were IgG negative and did not have a recorded CMV+ result ("CMV-negative"). The percentages of four CD8+ phenotypes (CD8+CD62L+CD45RA+; CD8+CD62L-CD45RA+; CD8+CD95+CD28-; CD8+CD95-CD28+) were significantly associated with baseline CMV status in longitudinal adjusted models; the trends over time differed by CMV category and/or treatment arm, with significant week*CMV status and/or week*treatment arm interactions. No differences were noted in CD4+ phenotype, activated CD8+ phenotype (HLA-DR+38+), or other populations (CD8+CD62L+CD45RA-%, CD8+CD62L-CD45RA-%, CD8+CD95+CD28+%, or CD8+CD95-CD28-%) by CMV status in multivariate models

Conclusions: In PHIV+ children with severe disease, changes in percentages of CD8+CD62L+CD45RA+, CD8+CD95-CD28+ (naïve), CD8+CD62L-CD45RA+ (terminally differentiated) and CD8+CD95+CD28- (senescent) were noted after switching to new multi-drug ART regimen depended on the children's CMV positivity and viremia status and treatment arm; the effect was independent of VL treatment response. Combined, these findings suggest an independent role of CMV co-infection in alterations in CD8+ T-cell senescence and confirm the effect of CMV in driving terminal differentiation and senescence in PHIV+ children.

BACKGROUND

Unlike HIV+ adults, whose ART-induced immune reconstitution is mainly driven by memory T-cells, in PHIV+ children it originates primarily from the naive T-cell pool and a rebound in thymopoiesis [1].

CMV replication may contribute to immune activation in co-infected HIV+ adults, and pharmacologic suppression of CMV may help reverse this process [2], but these findings may not be generalizable to PHIV+ children [3-6]

Given the high relevance and uniqueness of immune recovery patterns in PHIV+ children, it is important not only to examine the impact of CMV co-infection on activated CD4+ and CD8+ subsets, but also on other potentially relevant compartments.

OBJECTIVES

1. To examine longitudinal trends in percentages of activated (CD38+HLA-DR+), senescent (CD95+CD28-) and naïve (CD62L+45RA+) compartments of CD4+ and CD8+ T-cells by CMV positivity and viremia status in a cohort of PHIV+ children with severe disease following a therapeutic switch in HAART regimen
2. To explore longitudinal trends in percentages of additional CD4+ and CD8+ T-cell phenotypes by CMV positivity and viremia status based on permutations of expression of the following pairs of markers: CD38/HLA-DR, CD62L/CD45RA, CD95/CD28

METHODS

P366 was a partially randomized, open-label, multicenter 96-week treatment-algorithm study. P366 enrolled PHIV+ participants 6 months to 21 years old who, after receiving at least 8 weeks of unchanged continuous ART, had on two consecutive clinical visits at least one of the following features of severe disease: HIV-1 plasma VL >50,000 copies/mL; CD4+ count <200 cells/mm3, CD4% <15, or a 50% reduction in CD4% within 24 weeks of the start of the current ART regimen; growth failure; or CNS disease. The participants were required to be naïve to at least 2 drugs, of which 1 was nevirapine (NVP), nelfinavir (NFV) or ritonavir (RTV). Based on prior ART class experience, they were assigned a 4-drug ART regimen in which at least 2 agents were new and at least one of the new agents was nevirapine, nelfinavir or ritonavir. The primary aim of P366 was to determine the proportion of participants in the study treatment arms that had a reduction in VL following the switch and determine duration of the reduction. Enrollment occurred between May 1998 and January 2000 at 50 US sites.

METHODS

- **The present analysis included only PHIV+ P366 enrollees ≥1 year old with T-cell phenotype data and baseline stored plasma samples.**
- CMV IgG (ELISA, Genway Biotech Inc.) and CMV DNA (Realtime PCR, Abbott Molecular) testing was done on stored plasma samples. CMV status at entry was categorized into three groups:
  CMV+ viremic – detectable CMV DNA
  CMV+ non-viremic – IgG+ or any positive result assessed at study baseline (ie. blood culture, serology, PCR or antigen or urine culture)
  CMV-negative – no positive CMV results recorded
- Immunophenotyping of CD4+ and CD8+ T-cells was performed at study weeks 0 (baseline), 12, 20 and 40 using flow cytometry in laboratories that participated in the DAIDS Quality Assurance Program. Immune markers assessing for naïve (CD62L+CD45RA+; CD95-CD28+), activated (CD38+HLA-DR+), apoptotic (CD95+), terminally differentiated (CD62L-RA+) and senescent (CD28+CD95+) CD4+ and CD8+ T cells were evaluated.
- The association between baseline CMV status and 24 phenotype percentages at weeks 0, 12, 20, and 40 were assessed with linear mixed models, adjusted for the covariates in Table 1 and treatment group by week interaction.

RESULTS

One hundred and seven PHIV+ children enrolled in P366 and ≥1 year old who had T-cell phenotype data and baseline stored plasma samples available were included in the analysis. (Table 1)

Table 1: Participant Characteristics by CMV Status

Characteristic		CMV status: CMV+ viremic (N=15)	CMV+ non-viremic (N=52)	CMV- (N=40)	Total (N=107)	P
Age	1-5 years	11 (73%)	18 (35%)	19 (48%)	48 (45%)	0.43 [a]
	6-12 years	3 (20%)	28 (54%)	17 (43%)	48 (45%)	
	>12 years	1 (7%)	6 (12%)	4 (10%)	11 (10%)	
Gender	Male	4 (27%)	36 (69%)	19 (48%)	59 (55%)	0.01 [b]
	Female	11 (73%)	16 (31%)	21 (52%)	48 (45%)	
Race/ Ethnicity	Black Non-Hispanic	10 (67%)	26 (50%)	26 (65%)	62 (58%)	0.27 [b]
	Hispanic	5 (33%)	18 (35%)	8 (20%)	31 (29%)	
	White Non-Hispanic/Other	0 (0%)	8 (15%)	6 (15%)	14 (13%)	
ARV regimen at entry	HAART with NFV	3 (20%)	16 (31%)	10 (25%)	29 (27%)	0.89 [b]
	HAART without NFV	3 (20%)	12 (23%)	9 (23%)	24 (22%)	
	Not on HAART	9 (60%)	24 (46%)	21 (53%)	54 (50%)	
Treatment group	2 NRTIs + NVP/NFV	3 (20%)	10 (19%)	9 (23%)	22 (21%)	0.64 [b]
	2 NRTIs + NVP/RTV	5 (33%)	9 (17%)	7 (18%)	21 (20%)	
	1 NRTI + NVP/NFV/RTV	3 (20%)	17 (33%)	8 (20%)	28 (26%)	
	2 NRTIs + NFV/RTV	4 (27%)	16 (31%)	16 (40%)	36 (34%)	
HIV-1 VL response[1]	Response	4 (27%)	20 (38%)	20 (50%)	44 (41%)	0.37 [b]
	Partial response	2 (13%)	12 (23%)	6 (15%)	20 (19%)	
	Non-response	6 (40%)	15 (29%)	7 (18%)	28 (26%)	
	Missing	3 (20%)	5 (10%)	7 (18%)	15 (14%)	
HIV-1 RNA level (cp/ml) at entry, median (Q1,Q3)		79,725 (44,203, 157,089)	60,670 (27,031, 121,585)	57,598 (22,426, 152,534)	59,818 (24,807, 129,510)	0.52 [c]
CD4 percent at entry, median(Q1,Q3)		22 (12, 24)	17 (11, 24)	21.5 (9.0, 28.5)	19 (11, 27)	0.64 [c]

[1]Assessed as change in HIV viral load (VL) (log_{10}copies/mL) from average pre-entry/entry to average week 12/16, with "response" defined as decrease to <400 copies/mL at week 12/16, "partial response" defined as decrease of at least 0.75 log_{10}copies/mL, and "non-response" otherwise if non-missing
[a] Mantel Haenszel Chi-Square Test, [b] Chi-Square Test, [c] Kruskal-Wallis Test

RESULTS

Senescent T-cells
- CMV+ viremic patients had the highest mean CD4+CD95+CD28-% at all time points (Fig.1), but there were no significant differences by CMV status in the linear mixed model.
- Mean CD8+CD95+CD28-% was lowest in the CMV-negative group at all time points (Fig. 1). CD8+CD95+CD28-% was significantly higher at baseline for CMV+ viremic participants compared to CMV-negative participants (p=0.016, Table 2). The rate of change in CD8+CD95+CD28-% over time was significantly higher for CMV+ viremic and non-viremic compared to CMV- participants (p=0.010 and p=0.002, respectively).

Activated T-cells
- Mean CD4+CD38+HLA-DR+% was similar in the three CMV groups at baseline but highest in the CMV+ viremic group at all other time points (Fig.1)
- Mean CD8+CD38+HLA-DR+% was highest in the CMV+ viremic group and lowest in the CMV-negative group at all time points (Fig.1)
- Neither marker differed by CMV status in linear mixed models.

Naive T-cells
- Mean CD4+CD62L+CD45RA+% was similar in all three groups at baseline (Fig.1) and trends did not vary significantly by CMV status at baseline or over time in the linear mixed model.
- Mean CD8+CD62L+CD45RA+% was highest in the CMV-negative group at all time points (Fig. 1). CD8+CD62L+CD45RA+% was significantly lower at baseline for CMV+ non-viremic participants compared to CMV-negative participants (p=0.018, Table 2). The rate of change was significantly lower for CMV+ non-viremic vs. CMV-negative participants (p=0.008).

Other T-cell phenotypes
- Mean CD8+CD95-CD28+% was highest in the CMV-negative group at all time points (Fig. 1). CD8+CD95-CD28+% was did not differ by CMV status at baseline, but the rate of change was significantly lower in the CMV+ viremic (p=0.013) and non-viremic groups (p=0.002) compared to the CMV-negative group (Table 2).
- Mean CD8+CD62L-CD45A+% was lowest in the CMV-negative group at all time points (Fig. 1). CD8+CD62L-CD45A+% was significantly higher in the CMV+ non-viremic group than in the CMV-negative group at baseline (p=0.018) but trends did not vary significantly by CMV status over time (Table 2).
- None of the other T-cell phenotype percentages varied by CMV status in linear mixed models.

Figure 1: Mean T-cell Phenotype Percentages by CMV Status and Week

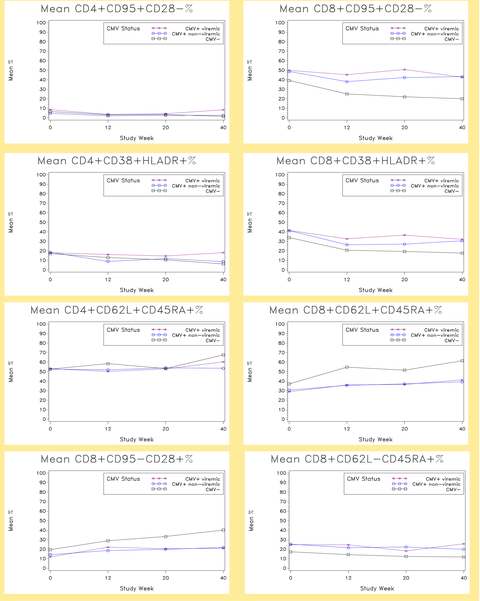


Table 2: Linear Mixed Model Estimates for Significant Markers vs. CMV Status

Covariate	CD8+CD95+ CD28-% Estimate	p	CD8+CD62L+ CD45RA+% Estimate	p	CD8+CD95- CD28+% Estimate	p	CD8+CD62L- CD45RA+% Estimate	p
Week	-0.20	0.05	0.30	<0.001	0.20	0.011	-0.05	0.45
CMV status		0.038		0.029		0.33		0.049
CMV+ viremic vs. CMV-	12.38	0.016	-8.79	0.06	-4.89	0.24	5.51	0.17
CMV+ non-viremic vs. CMV-	5.49	0.11	-7.59	0.018	-3.47	0.22	6.71	0.018
Week *CMV status		0.003		0.025		0.004		0.38
Week *CMV+ viremic	0.43	0.010	-0.23	0.10	-0.32	0.013	0.16	0.17
Week *CMV+ non-viremic	0.33	0.002	-0.24	0.008	-0.25	0.002	0.02	0.75

[1]Estimates are adjusted for covariates in Table 1 and treatment group by week interaction

CONCLUSIONS

1. In PHIV+ children with severe disease, changes in percentages of CD8+CD62L+CD45RA+, CD8+CD62L-CD45RA+, CD8+CD95+CD28- and CD8+CD95-CD28+ after switching to new multi-drug ART regimen depended on the children's CMV positivity and viremia status.
2. In HAART-treated PHIV+ children with history of severe disease, the recovery of naïve CD8+ T-cell compartment and decreases of senescent and apoptosis-prone CD8+ T-cell compartments might be adversely affected by CMV co-infection. This may occur even in the circumstance of successful HIV viremia suppression.
3. If confirmed by future studies, these findings may have implications for therapeutic interventions targeting CMV co-infection and/or replication in PHIV+ children

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