Acute cytomegalovirus infection is associated with increased frequencies of activated and apoptotic-vulnerable T cells in HIV-1 infected infants

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ABSTRACT

Cytomegalovirus (CMV) co-infection is associated with infant HIV-1 disease progression and mortality. In a cohort of Kenyan HIV-infected infants, the frequency of activated (CD38^HLA-DR^) and apoptosis-vulnerable (CD95^-Bcl-2^-) CD4^+ and CD8^- T cells increased substantially during acute CMV infection. The frequency of activated CD4^+ T cells was strongly associated with both concurrent CMV co-infection (p=0.001) and HIV-1 viral load (p=0.05). The frequency of apoptosis-vulnerable cells was also associated with CMV co-infection in the CD4 (p=0.02) and CD8 T cell subsets (p<0.001). Similar observations were made in HIV-exposed uninfected infants. CMV-induced increases in T cell activation and apoptosis could contribute to the rapid disease progression in co-infected infants.
Acute infant HIV-1 infection is characterized by very high HIV-1 viral loads (25, 28), rapid CD4 depletion, and high rates of mortality (1, 22, 24). Cytomegalovirus (CMV) co-infection is associated with more rapid HIV-1 progression in children (10, 17, 23); and in adults, plasma CMV DNA is associated with survival time (6, 12, 34). In resource-poor settings, where CMV is often acquired during infancy (21, 32), a large population of children undergo simultaneous primary HIV-1 and CMV infection (32).

CD8+ T cell activation has previously been shown to accompany acute CMV infection in healthy Gambian infants (21). Since T cell activation is a strong predictor of HIV-disease progression (7, 13, 14, 27), we hypothesized that the acquisition of CMV during primary HIV-1 infection may accelerate infant disease progression by increasing frequencies of activated cells. In this report we describe longitudinal changes in activated and apoptosis-vulnerable T cells during acute CMV infection in HIV-infected and HIV-exposed uninfected infants (HIV-EU).

**Study participants and specimens.** The primary cohort involved follow-up of 474 Kenyan infants from 1999-2003, detailed elsewhere (15, 19). This study was conducted before antiretroviral therapy (ART) became widely available in Kenya, and women and infants received ART only for PMTCT. Serial infant blood specimens were collected at delivery, months 1, 3, and quarterly thereafter; HIV-EU infants exited at 1 year and HIV-infected infants exited at 2 years. Plasma specimens were used for measurement of HIV-1 (11) and CMV viral load (20, 32).

Infant HIV-1 infection was diagnosed as the first detection of HIV-1 using dried blood spot PCR for HIV-gag (8) or plasma HIV-1 RNA viral load, whichever appeared first. CD4 counts were performed on freshly isolated blood using TriTest antibodies (BD Biosciences, USA) and flow cytometry.
CMV viral loads were measured in a subset of 64 infants (32, 33); the current report involves a sample of 19 HIV-infected and 6 HIV-exposed uninfected (HIV-EU) infants selected by availability of cryopreserved PBMC (Table S1). CMV DNA was detected in the plasma of all but one infant.

**Acute CMV infection is associated with an expansion of activated and apoptosis-vulnerable T cells in HIV-infected infants.** An increase in CD8+ T cell activation has been observed in HIV-negative children and transplant recipients with primary CMV infection (18, 21, 26, 29, 35).

Cellular activation contributes to HIV-1 pathogenesis by a number of mechanisms (reviewed in (9, 16)), including depletion of T cells via activation induced cell death (AICD). Apoptosis is a hallmark of HIV-infection; CD95 (Fas) is upregulated during HIV-1 infection, and its expression increases during disease progression (2-4, 30). We measured frequencies of activated and apoptosis-vulnerable CD4+ and CD8+ T cells. Peripheral blood mononuclear cells (PBMC) were thawed and stained with CD3-Pacific Blue (UCHT1, Dako, UK), CD4-APC-Cy7 (RPA-T4, Pharmingen, UK), CD8-PE-Cy7 (RPA-T8, Pharmingen), CD38-PE (AT13/5, Serotec, UK), and HLA-DR-APC (TU36, Pharmingen) antibodies and analyzed with multicolor flow cytometry, using standard methods described elsewhere (31). Activated CD3+CD4+ and CD3+CD8+ T cells were defined as CD38+HLA-DR+ (Figure 1A). Cells expressing CD95 which had down-regulated expression of Bcl-2 were considered “apoptosis-vulnerable” cells likely to undergo AICD (5, 36) (CD95+Bcl-2−, Figure 1B).

Figure 2A&B show longitudinal frequencies of activated and apoptosis-vulnerable T cells in infants grouped by timing of HIV-1 infection. In HIV-infected infants, the frequency of activated and apoptosis-vulnerable CD4+ and CD8+ T cells increased concurrently with the first detection of CMV DNA (Figure 2C, p<0.05 for baseline vs acute). This increase was also
observed in HIV-EU infants, although it occurred more gradually in the CD8 subset (Figure 2D, p=0.05 for baseline vs post-infection). The frequency of activated (median 20%, IQR=20-47) and apoptosis-vulnerable (median 41%, IQR=7.7-27) CD8+ T cells we measured in HIV-EU is consistent with an earlier study in HIV-unexposed Gambian infants which measured high levels of activated (28% HLA-DR+) and apoptosis-vulnerable (56% Bcl-2-) CD8+ T cells during acute CMV infection (21).

HIV-1 load also increased by an average 0.52 log10 copies/ml (+SD=1.1; p = 0.03) between baseline and acute CMV infection. Although CMV co-infection was associated with more rapid HIV-1 progression and higher mortality in an American cohort, CMV co-infection was not associated with higher HIV-1 viral loads (17). To determine whether HIV-1 viral load was affected by acute CMV infection in this Kenyan cohort, we compared mean HIV-1 viral loads between children who were CMV-infected and CMV-negative at 1 month of age. Consistent with previous findings, there was no difference in CMV viral load between infants with CMV co-infection (mean 6.2 log10 copies/ml ±SD 0.91) and those with HIV-1 infection alone (6.5 log10 copies/ml ±SD 0.77, p=0.4). It is thus likely that the increase in HIV-1 viral load we observed during acute CMV infection was not likely due to CMV, but rather was coincidental to acute HIV-1 infection.

At 1 month of age, HIV-1/CMV co-infected infants have a higher frequency of activated CD4+ T cells. Because T cell activation is predictive of long-term risk of HIV-1 disease progression, we compared frequencies of activated and apoptosis-vulnerable cells at 1 month of age (Figure 3). HIV+CMV+ had a higher frequency of activated CD4+ T cells compared to HIV+CMV- infants (median 3.0% vs 1.6%, respectively, Mann-Whitney U test, p=0.03). In the CD8+ T cell subset, HIV+CMV+ and HIV+CMV- infants both had higher frequencies of activated and
apoptosis-vulnerable cells at 1 month compared to HIV-CMV- infants (p<0.05 for each comparison).

CMV co-infection is associated with frequencies of activated T cells. Generalized estimating equations (GEE) were used to determine predictors of activated and apoptosis-vulnerable cell frequencies; outcomes were continuous and used the identity link and Gaussian errors (Table S2A). All models used an exchangeable correlation matrix and robust standard errors. GEE beta-coefficients were used to predict longitudinal frequencies of activated and apoptosis-vulnerable T cells in the presence and absence of CMV infection (Figure 4).

Figure 4A shows that in the presence of CMV co-infection, HIV-infected infants are predicted to have substantially higher frequencies of activated T cells. In HIV-infected infants, the frequency of activated CD4+ T cells was predicted by CMV co-infection at the concurrent visit (Table S2, p=0.001) and also HIV-1 viral load (p=0.05). The effect of CMV co-infection was further enhanced by the level of HIV-1 viral load (interaction term, p<0.001), suggesting a synergistic effect of CMV co-infection and HIV-1 viral load on CD4+ T cell activation. The frequency of activated CD8+ T cells was dependent upon both the presence of CMV co-infection (p=0.004) and CMV viral load (p=0.009), but was not affected by HIV-1 viral load.

In HIV-EU infants, CMV co-infection was also associated with the frequency of activated CD4+ T cells (p=0.01).

CMV co-infection is associated with frequencies of apoptosis-vulnerable T cells. Figure 4B shows that in the presence of CMV co-infection, HIV-infected infants are predicted to have substantially higher frequencies of apoptosis-vulnerable T cells. In HIV-infected infants, the frequency of apoptosis-vulnerable CD4+ and CD8+ T cells was associated with the presence of CMV co-infection (Table S2B, p=0.02 and p<0.001, respectively). Interestingly, HIV-1 viral
load was not a significant predictor of apoptosis-vulnerable CD4$^+$ or CD8$^+$ T cells. We may have failed to find this association due to correlation between HIV-1 and CMV viral load, and because T cell activation is in the causal pathway between HIV and/or CMV viral load and apoptosis. The frequency of apoptosis-vulnerable CD8$^+$ T cells was also associated with the frequency of activated CD8$^+$ T cells ($p=0.01$).

In the HIV-EU controls, we observed a strong association between the frequency of activated T cells and apoptosis-vulnerable T cells in both the CD4 and CD8 subsets, suggestive of AICD. CMV co-infection was significantly associated with apoptosis-vulnerability only in the CD8 subset ($p=0.05$).

In conclusion, we found that acute CMV infection was accompanied by substantial increases in the frequency of activated and apoptosis-vulnerable T cells, and that levels of activated and apoptosis-vulnerable T cells during acute HIV-1 infection were largely determined by the presence of CMV co-infection. Furthermore, HIV-1 viral load and CMV co-infection synergistically increased the frequency of activated CD4$^+$ T cells, suggesting CMV co-infection may play an important role in CD4$^+$ T cell depletion during acute infant HIV-1 infection. These data support the hypothesis that CMV-induced T cell activation and Fas-mediated apoptosis potentially contribute to the increased HIV-1 disease progression observed in CMV co-infected infants. As ART becomes more widely accessible to this population for both PMTCT and treatment, it will be important to determine the impact of maternal and infant ART on CMV epidemiology and pathogenesis.
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FIGURE LEGENDS

Figure 1. Activated and apoptosis-vulnerable T cells in infants with HIV-1 infection, CMV infection, and HIV-1/CMV coinfection. Representative A) CD38 and HLA-DR and B) CD95 and Bcl-2 staining from four infants categorized by HIV-1 and CMV infection status. All plots show data at 1 month of age, with the exception of subject 081, whose data are from month 3 (all HIV-EU infants first tested CMV DNA positive at 3 months).

Figure 2. Changes in frequencies of activated and apoptosis-vulnerable T cells during acute CMV infection. Connected lines show individual infant trajectories of A) activated and B) apoptosis-vulnerable CD4+ and CD8+ T cells in three groups of infants (HIV-infected in utero, HIV-infected peripartum and HIV-exposed uninfected). Time=0 corresponds to the first detection of CMV DNA. C&D) Formal statistical comparison of frequencies of activated and apoptosis-vulnerable cells at baseline (last CMV negative visit), acute CMV infection (first CMV positive visit), and post-acute infection (first visit after acute) for HIV-infected and HIV-EU infants. Individual infants are shown by gray lines and the solid back median spline line is overlaid. P values for paired sign-rank test. Note: y-axes are shown on a different scale for CD4+ and CD8+ T cell subsets for clarity of data presentation.

Figure 3. Comparisons of activated and apoptosis-vulnerable T cells at 1 month of age by HIV/CMV co-infection. Median and interquartile ranges are shown for infants grouped by co-infection at 1 month of age: negative for both viruses (HIV-CMV-), infected with HIV only (HIV+CMV-), or infected with both viruses (HIV+CMV+). Because all HIV-exposed uninfected infants first tested positive for CMV at 3 months of age, there is no HIV-CMV+ group to display at month 1.
Figure 4. Longitudinal models of T cell activation and apoptotic-vulnerability. Beta-
coefficients from GEE models were used to create predictive models using the general linear
model (see Table S2). Scatter plots and fitted curves show observed data and predictive models
for the outcomes of A) % activated cells and B) % vulnerable cells in HIV-infected and HIV-
exposed uninfected infants. HIV-EU exited the study at 12 months, HIV-infected infants were
followed for an additional year. No HIV-EU infants acquired CMV before 3 months, so observed
data and CMV-infected predictive models are not plotted for the month 0 and 1 time-points. P
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