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Acute cytomegalovirus infection in Kenyan HIV-infected infants

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Abstract

Objective—Cytomegalovirus (CMV) coinfection may influence HIV-1 disease progression during infancy. Our aim was to describe the incidence of CMV infection and the kinetics of viral replication in Kenyan HIV-infected and HIV-exposed uninfected infants.

Methods—HIV-1 and CMV plasma viral loads were serially measured in 20 HIV-exposed uninfected and 44 HIV-infected infants born to HIV-infected mothers. HIV-infected children were studied for the first 2 years of life, and HIV-exposed uninfected infants were studied for 1 year.

Results—CMV DNA was detected frequently during the first months of life; by 3 months of age, CMV DNA was detected in 90% of HIV-exposed uninfected infants and 93% of infants who had acquired HIV-1 *in utero*. CMV viral loads were highest in the 1–3 months following the first detection of virus and declined rapidly thereafter. CMV peak viral loads were significantly higher in the HIV-infected infants compared with the HIV-exposed uninfected infants (mean 3.2 versus 2.7 $\log_{10}$ CMV DNA copies/ml, respectively, $P = 0.03$). The detection of CMV DNA persisted to 7–9 months post-CMV infection in both the HIV-exposed uninfected (8/17, 47%) and HIV-infected (13/18, 72%, $P = 0.2$) children. Among HIV-infected children, CMV DNA was detected in three of the seven (43%) surviving infants tested between 19 and 21 months post-CMV infection. Finally, a strong correlation was found between peak CMV and HIV-1 viral loads ($\rho = 0.40$, $P = 0.008$).

Conclusion—Acute CMV coinfection is common in HIV-infected Kenyan infants. HIV-1 infection was associated with impaired containment of CMV replication.

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Keywords

acute infection; cytomegalovirus; opportunistic infection; paediatric HIV; pathogenesis

Introduction

Cytomegalovirus (CMV) is a major viral cause of congenital disease globally, affecting 0.2–3% of live births in high-income and 4–14% in low-income regions [1–6]. CMV prevalence varies among populations according to socioeconomic conditions [7], with poorer communities having a relatively higher prevalence and earlier incidence. A recent estimate reported approximately 54% of American adults in their thirties to be CMV seropositive [8], whereas approximately 85% of Gambian infants acquire CMV before they are a year old [9].

Although CMV does not typically cause disease in healthy individuals, the virus has clinical significance during primary infection or reactivation in the immunosuppressed. CMV infection is associated with HIV-1 disease progression and mortality in adults [10–13]. In the absence of HAART, patients with CD4 cell counts below 100 cells/μl are at a high risk for CMV-associated retinitis and gastrointestinal and neurologic disease [14–17]. In United States cohorts, CMV coinfection has been noted in up to 40% of HIV-infected infants during the first year of life and is associated with an approximately 2.5-fold increased risk of disease progression [18,19]. In Kenya, we recently reported that the detection of maternal CMV DNA in the blood near the time of delivery was associated with a three to four times increased rate of mortality in HIV-infected infants [20]; this relationship remained significant after controlling for other strong predictors of infant mortality, including maternal CD4 cell count, CD4 cell percentage, HIV-1 RNA viral load and maternal death.

If CMV presents a significant risk factor for HIV-1 disease progression, its impact may be particularly important in African children where both viruses are commonly acquired in infancy [2,21]. In order to understand the mechanisms that underlie the relationship between CMV coinfection and rapid HIV-1 disease progression, it is first necessary to determine the incidence of CMV infection among HIV-infected infants and to describe its natural history. Although risk factors associated with vertical CMV transmission are well defined, very few studies [22,23] have measured CMV replication quantitatively in infants, and only one longitudinal study [24] has described infant CMV viral load in the setting of HIV-1. The purpose of our study was to describe the incidence and timing of CMV infection and the kinetics of CMV viral replication in HIV-infected and HIV-exposed uninfected Kenyan infants.

Methods

Participants and study design

Study protocols were approved by the Ethics Review Committee of Kenyatta National Hospital and the Institutional Review Board of the University of Washington. A cohort of infants born to HIV-infected women was used to study acute infant CMV infection. As part of a larger cohort study of perinatal HIV-1 transmission, HIV-infected pregnant women were recruited in Nairobi between 1999 and 2003 [25,26].

The women received short-course zidovudine for prevention of HIV-1 transmission [27]. Mothers and infants were followed during pregnancy, delivery and for 1–2 years postpartum, during which time serial blood specimens were obtained in pregnancy, at delivery and months 1, 3, 6, 9, 12, 15, 18, 21 and 24 postpartum. Sixty-four infants were selected from the larger cohort based on survival to at least 3 months of age and the availability of a plasma specimen

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by 1 month of age. Infants were followed until death or exit from the study at 1 year (HIV-exposed uninfected) or 2 years of life (HIV infected).

HIV-1 diagnosis and quantification

Diagnosis of infant HIV-1 infection was made using PCR amplifying HIV-1 gag DNA from dried blood spotted onto filter paper as previously described [28]. HIV-1 RNA viral loads were measured using the Gen-Probe assay [29]. HIV-1 infection was defined as the detection of either HIV-1 DNA or RNA; the timing of HIV-1 infection was estimated as the midpoint between the last HIV-negative test and the first HIV-1-positive test. Infants were grouped according to first HIV-1 detection: *in utero* (within 48 h of birth, $n = 15$), peripartum (uninfected at birth, infected at 1 month, $n = 16$) or late (infected after 1 month, $n = 13$). In the 'late' infection group, estimated infection times were: 2 months (six infants), 2.5 months (one infant), 4.5 months (one infant), 5 months (one infant), 6 months (one infant), 7.5 months (two infants) and 10.5 months (one infant). Peak and set-point HIV-1 viral load were used to describe the dynamics and control of HIV-1 replication during early infection. We defined peak HIV-1 viral load as the highest measurement in the first 6 months after infection; HIV-1 set-point was defined as the first viral load observation measured at least 6 weeks after the peak [30].

Cytomegalovirus viral load measurements

Cord blood was used to diagnose in utero CMV transmission. Following delivery of the placenta, umbilical cords were clamped in two locations and swabbed to remove maternal blood. Blood was then collected with a syringe and transferred to EDTA Vacutainer tubes (Becton Dickinson Diagnostics, Franklin Lakes, New Jersey, USA). At all other time-points, venous blood was collected. Viral nucleic acids were extracted from 50–200 μl of plasma using the Qiagen UltraSens virus extraction kit (Qiagen, Valencia, California, USA). Quantitative PCR was used to detect the glycoprotein B gene [31], and copy number was determined with the aid of a standard curve derived from known quantities of cloned amplicon DNA. Each individual's viral load was determined by calculating the mean of three replicate reactions. The lower limit of detection was one copy per reaction. Negative (no DNA detection) and indeterminate (less than one copy per reaction) PCR assays were not included in calculations of median or peak viral load and were categorized as negative for CMV DNA.

Indeterminate PCR assays were assigned a value equivalent to the midpoint between the limit of detection and zero for longitudinal modelling but were not included in the calculation of peak CMV viral load. Peak CMV viral load was defined as the highest viral load observed for each CMV-infected infant during the first 6 months after infection.

Statistical analysis

STATA SE version 9 for Macintosh (STATA Corp., College Station, Texas, USA) was used for the statistical analysis. Viral loads were base 10 log-transformed ($\log_{10}$). The *t*-test was used to compare continuous variables, and Fisher's exact tests were used to compare proportions. S-Plus (S-Plus 2000; Mathsoft, Inc., Seattle, Washington, USA) was used to create non-parametric smoothers for CMV DNA over time using Freidman's super smoother. Area under the curve (AUC) for each child's log viral load from 3 to 12 months of life was estimated using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA). The *t*-test was used to compare mean AUC of longitudinal viral loads between groups of infants. Spearman's rank correlation coefficient was used to describe the correlation between HIV-1 and CMV viral load. All *P* values reported are for two-tailed tests.

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Results

Patient characteristics

Median maternal age at enrolment was 25 years [interquartile range (IQR) = 22–29 years], and median parity was 1 (IQR = 1–2). Characteristics of the infants studied are shown in Table 1. HIV-infected infants were followed for 24 months; infants with no HIV-1 detection exited the study at 12 months. As previously reported, HIV-1 viral loads and mortality were very high among HIV-infected infants in the cohort, and there were 23 infant deaths among the selected patients [30,32].

Cytomegalovirus acquisition occurs primarily during the first 3 months of life in Kenyan HIV-exposed infants

HIV-1 and CMV viral loads were measured serially in 44 HIV-infected and 20 HIV-exposed uninfected infants (390 concurrent HIV-1 and CMV viral load measurements). Plasma specimens were available for CMV DNA assessment at birth from 51 infants and by 1 month of age from all 64 infants. CMV DNA was detected in the cord blood plasma of 29% (4/14) of HIV-infected newborns and 2.7% (1/37) of infants who were HIV-1 RNA negative at birth ($P = 0.02$). None of the infants with detection of CMV DNA at birth had overt clinical evidence of congenital CMV disease.

In both the HIV-infected and HIV-exposed uninfected infants, the majority of CMV infections occurred during the first 3 months of life (Table 2). By 3 months of age, CMV DNA had been detected in 90% of HIV-exposed uninfected infants, 93% of infants who were HIV infected at birth, 75% of infants who acquired HIV-1 peripartum and 85% of infants who acquired HIV-1 infection through late breast milk transmission. Only three infants in the study group had no CMV DNA detection during follow-up: two of these infants completed follow-up and one infant died at 3.5 months of age.

Cytomegalovirus DNA detection in HIV-unexposed infants

A small group of HIV-uninfected women and their infants ($n = 13$) with similar demographics to the larger study were examined to determine the prevalence of CMV in the absence of HIV-1. Of 13 HIV-negative women screened at delivery, 13 (100%) were CMV seropositive but none had detectable CMV DNA. Over 6 months of follow-up, CMV DNA was detected in six of 13 (46%) infants born to these mothers.

Cytomegalovirus DNA is commonly detected in plasma during the first 1–2 years of infection

CMV DNA viral loads were measured longitudinally during follow-up. Although there was heterogeneity between individual infants during acute CMV infection, the overall pattern was a peak at the first detection of CMV or at the following measurement, followed by a decline (Fig. 1). Among the group of infants with late breast milk acquisition of HIV-1 who acquired CMV before HIV-1, secondary peaks in CMV viral load were sometimes observed concurrent to the first detection of HIV-1 RNA (one infant who became CMV DNA positive at 1 month and three infants who became CMV positive at 3 months).

Infant HIV-1 infection and cytomegalovirus replication

To determine whether HIV-1 infection compromised control of CMV replication, we compared peak CMV viral loads between HIV-infected and HIV-exposed uninfected infants (Fig. 2a). To avoid assessing cases in which infants acquired HIV-1 several months after having acquired CMV, we excluded the late HIV-infected infants in this comparison. We detected higher peak CMV viral loads among the infants with HIV-1 infection [mean 3.2, standard error (SE) 0.15]

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compared with the HIV-exposed uninfected infants (2.7 SE, 0.15 $\log_{10}$ CMV D-NA copies/ml, $P = 0.03$).

We also examined control of CMV replication by comparing the decline in CMV viral loads between HIV-infected and HIV-exposed uninfected infants (Fig. 2b). To control for differential timing of CMV and HIV infection, we restricted the analysis to children who first became CMV DNA positive at 3 months of age and excluded the late HIV-1 acquisition group. In both HIV-infected and HIV-exposed uninfected infants, CMV viral load declined steadily from infection to 12 months of age. However, the mean AUC for CMV replication had a trend to be higher among the HIV-infected than the HIV-exposed uninfected group ($P = 0.09$), suggesting a trend for more rapid reduction of CMV viral load in the HIV-exposed uninfected infants.

There was also a significant correlation between peak CMV and peak HIV-1 viral load (correlation coefficient $\rho = 0.40$, $P = 0.008$, Fig. 2c). Peak CMVand HIV-1 set-point viral load were also correlated in the 37 HIV-infected infants with HIV-1 set-point measurements ($\rho = 0.34$, $P = 0.04$, data not shown).

Persistence of cytomegalovirus DNA replication during the 1–2-year postinfection period

Although CMV viral loads were typically lower in the months following acute CMV infection, CMV DNA was commonly detected at several months after infection in both the HIV-infected and HIV-exposed uninfected infants (Table 3). As infants were evaluated every 3 months, we examined CMV DNA detection in 3-monthly intervals after first CMV DNA detection. Infants were counted once in each time interval; if an infant was tested more than once within the interval (five infants), only the test result at the later visit is reported. Between 7 and 9 months after the first detection of CMV DNA, CMV DNA was detected in 13 of 18 (72%) HIV-infected and eight of 17 (47%) HIV-exposed uninfected infants ($P = 0.2$). Among the HIV-infected infants, who were followed for an additional year, CMV DNA was detected in seven of 10 (70%) surviving infants who were tested between 13 and 15 months post-CMV infection and in three of seven (43%) infants tested between 19 and 21 months post-CMV infection.

Discussion

The current study highlights the extremely high incidence of CMV infection in Kenyan HIV-infected and exposed infants. CMV was almost universally acquired during the first year of life in the cohort, irrespective of HIV-1 status. HIV-1 infection altered the kinetics of CMV replication; CMV levels peaked at higher levels and had a trend to decline more slowly in HIV-infected infants. We found persistent detection of CMV DNA to be common in both HIV-1-infected and HIV-exposed uninfected infants in the 7–12-month postinfection period, suggesting incomplete containment of CMV replication by both HIV-infected and HIV-exposed uninfected infants. Finally, peak CMV and HIV-1 viral loads were highly correlated, suggesting that similar factors may affect the replication of both viruses.

CMV was acquired rapidly in this cohort. The cumulative incidence of CMV we observed at 6 months of age (81–93%) is higher than has been documented in healthy Gambian children (~53%) [2] and HIV-1-exposed (~15–20%) and HIV-infected children (~30–40%) in the United States cohorts of similar age [18,33]. Early CMV incidence in our cohort was probably due to the combination of maternal HIV-1 infection and prevalent breastfeeding and is likely to reflect infant CMV prevalence in similar African cohorts of breastfeeding HIV-infected mothers. We also observed a high frequency of in utero CMV transmission in the cohort. Increased rates of congenital CMV have previously been noted in HIV-infected newborns (~4–20%) [19]. In the current study, newborns with HIV-1 infection were more likely to have CMV at birth compared with HIV-exposed uninfected newborns. Several mechanisms may explain this observation; HIV-infected mothers who transmitted HIV-1 *in utero* would be

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expected to be more immunosuppressed, and therefore at increased risk of CMV transmission. Immunosuppression during foetal HIV-1 infection may result in a higher risk of CMV acquisition *in utero*. Alternately, foetal CMV coinfection may facilitate HIV-1 acquisition *in utero*.

Serial CMV viral load measurements enabled us to describe the kinetics of CMV replication in HIV-infected and HIV-exposed uninfected infants. We observed a rapid decline in CMV viral load following its first detection, followed by persistent or transient CMV detection. Low-level systemic CMV replication continued for many months after infection; CMV DNA could be detected in approximately 44% of the HIV-infected children tested after 16–18 months of infection. This frequency of detection is somewhat higher than that observed by Revello *et al*. [23] who measured CMV in the lymphocytes of approximately 25% of congenitally infected infants at more than 4 months of age. This long period of systemic CMV replication contrasts with reports from adults undergoing primary CMV infection, in which CMV DNA typically becomes undetectable in blood 6 months after infection [34,35]. This extended period of CMV replication may be explained by differences in cellular immune responses generated by infants and adults. Although infant CD8 T cells appear to be similar to adult T cells in their ability to expand and secrete interferon-gamma (IFNγ) [36], the infant CD4 cell response to CMV appears to be qualitatively and quantitatively different. Markedly lower T-helper cell 1 (Th1)-type CD4 cell responses have been reported in children compared with adults [37], and the clearance of viruria occurs concurrently with an increase in CMV-specific lymphoproliferation responses [38,39]. HIV-induced dysregulation of immune responses and destruction of CD4 T cells may further contribute to the preexisting age-related deficit in infant CMV-specific CD4 cell responses. Although it is beyond the scope of our current data to draw such conclusions, one may speculate that HIV-induced impairment of infant CMV-specific CD4 cell responses may contribute to the elongated period of systemic CMV replication observed in our study.

HIV infection was associated with higher peak CMV viral loads and a trend for a slower reduction in CMV viral load during the postacute phase. Unfortunately, we were unable to examine the relationship between CD4 cell counts and CMV viral load, but we speculate that impaired containment of CMV during infancy is most likely due to immunosuppression. Although overt signs of CMV disease were not noted in any of the children under study, we cannot rule out a contribution of CMV to the high rate of infant mortality in this cohort. CMV can cause a varied spectrum of diseases, including gastrointestinal illness, which was a major cause of death in the cohort [32].

In our study, peak CMVand HIV-1 viral load were highly correlated. In HIV-1-infected adults, correlations between CMV and HIV-1 viral load have been reported in the blood [12] and breast milk [40], and HIV-1 shedding in the cervix is more frequently detected if CMV DNA is detected in the same compartment [41]. In vitro studies have shown interactions between the viruses on a cellular level [13], and it is likely that local inflammation also fuels the replication of both viruses. Although the ex vivo data collected by us and others suggest an association between CMV and HIV-1 replication, it is at present impossible to infer a causal relationship or the direction of any such relationship. In the adult studies, the appearance of CMV in the blood is likely a result of reactivation of latent infection during severe immunosuppression, whereas the infants in our study were undergoing an acute CMV infection during periods of very high HIV-1 viraemia. The correlation between peak HIV-1 and CMV viral load likely suggests that similar host factors may affect the containment of both viruses.

Our study has some limitations. Infant urine specimens were not collected in the base cohort study, so virus culture from urine was not available as the gold standard method for diagnosing in utero CMV transmission. It is thus possible that we have underestimated the true incidence

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of in utero CMV transmission. Additionally, using HIV-1-exposed uninfected infants as controls likely underestimates the effects of HIV-1 infection on CMV containment. This group provided a convenient comparison group for our study, but HIV-exposed uninfected children are known to differ from HIV-unexposed children in terms of immunological development, morbidity and mortality [42]. Finally, we were unable to examine the effect of CMV on HIV-1 viral replication or mortality due to the very high incidence of CMV. A prospective trial designed with greater statistical power would be necessary to address the relationship between CMV infection, CMV viral load and mortality in HIV-infected Kenyan infants.

Conclusion

Acute concurrent CMV and HIV-1 infection occurs frequently in children born to HIV-infected Kenyan women. A high incidence of coinfection, impaired CMV containment, persistent CMV DNA detection and a correlation between CMV and HIV-1 peak viral loads suggest that CMV may play an important role in paediatric HIV-1 in this region. These results emphasize the urgent need for a CMV vaccine in sub-Saharan Africa. A recent clinical trial [43] of a recombinant glycoprotein B vaccine reported 50% efficacy against CMV infection in CMV-seronegative women, offering encouragement for the control of CMV infection in Kenya and other resource-poor settings.

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J.S., T.D., A.I., V.E., S.R.-J. and G.J.-S. conceived and designed the nested CMV study, and G.J.-S., B.R., E.O., D.M.-N. and B.L.-P. conceived and designed the parent study, which was designed to examine the correlates of maternal and infant disease/progression and mortality. J.O. and S.E. participated in the design of the virology assays and interpreted the virologic data. B.R. and J.S. performed the statistical analyses. All authors have participated in manuscript revisions and approved the final version.

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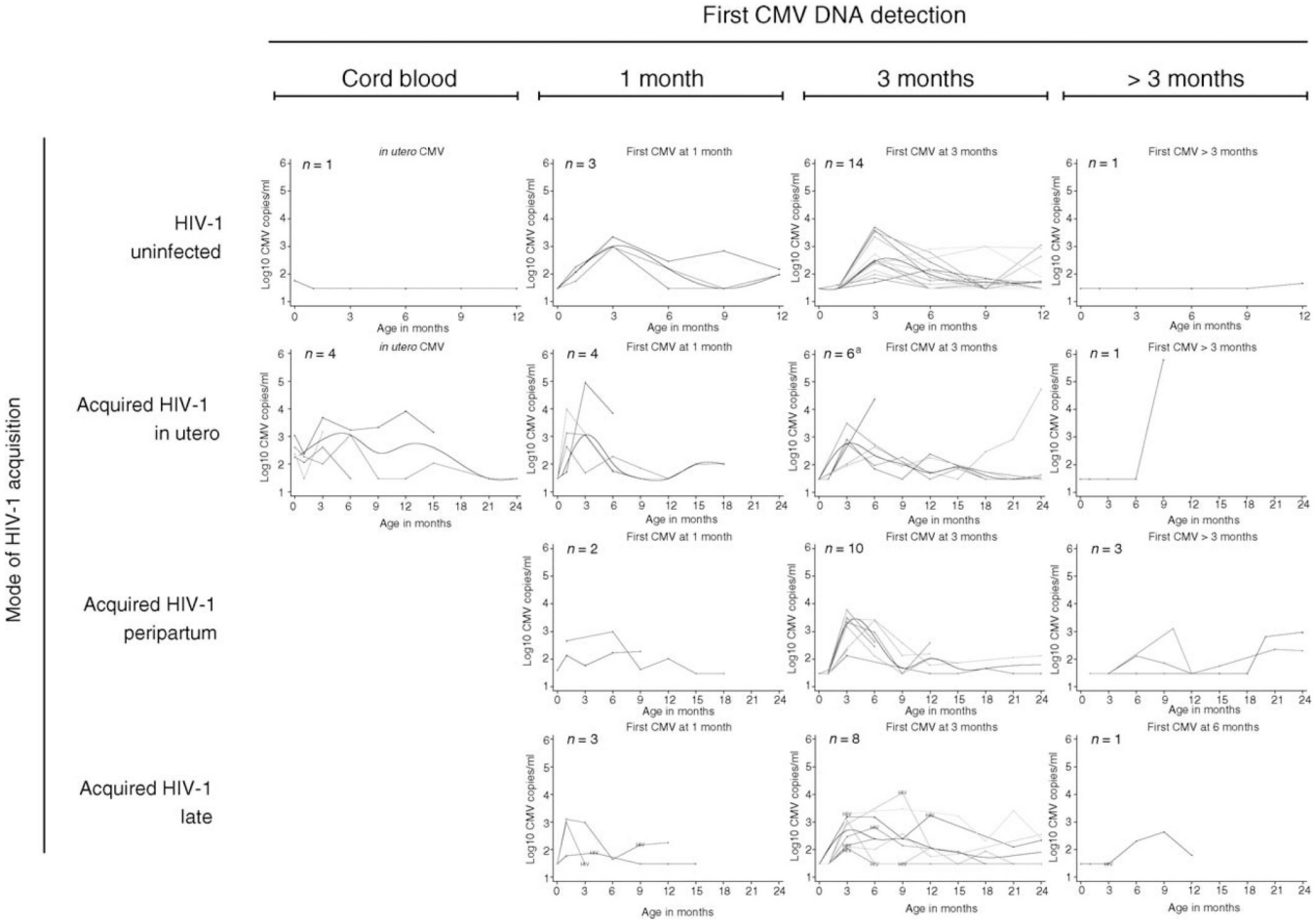

Fig. 1. The kinetics of cytomegalovirus viral load during acute infection in HIV-exposed infants

Infants are grouped by mode of HIV-1 acquisition (rows) and timing of first CMV detection (columns). Individual infants are represented by gray lines, when a sufficient number of cases were present to fit a model a median spline was added (black line). In the infants with late HIV-1 acquisition, the time of first HIV-1 detection for each infant is indicated by the point labelled 'HIV'. *n*, the number of infants in each group. [a]Includes one infant with first CMV detection at 2 months. CMV, cytomegalovirus.

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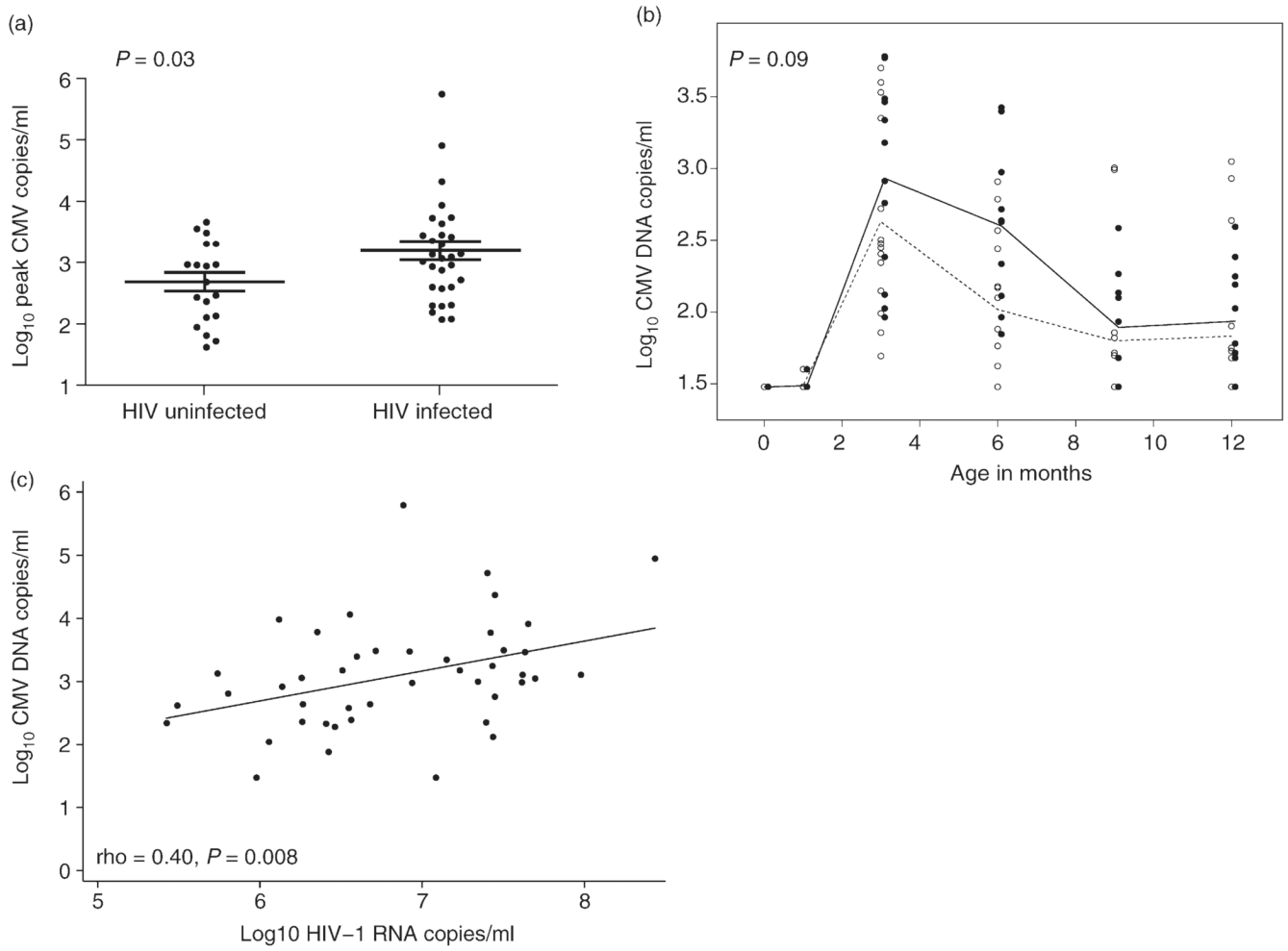

Fig. 2. HIV-1 infection and cytomegalovirus replication

(a) Mean peak CMV viral loads are shown for HIV-infected and HIV-exposed uninfected infants. Excludes infants who acquired HIV-1 after 1 month of age. Middle lines show group means and whiskers show standard error of the mean. (b) CMV viral loads are shown for HIV-infected and HIV-exposed uninfected infants. To control for age, differential timing of HIV/CMV infection and mortality, data are restricted to infants who were first CMV positive at 3 months of age and survived 12 months of follow-up. Infants with late HIV-1 acquisition were excluded from this figure. Solid line and solid circles show 15 infants with HIV-1, dashed line and open circles show 14 HIV-1-uninfected infants. Circles show individual infant measurements and lines show fitted median splines. (c) Scatter plot shows peak CMV and HIV-1 viral loads and linear regression line. CMV, cytomegalovirus.

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Table 1

Patient characteristics.

		Mode of HIV acquisition		
	HIV-exposed uninfected	***In utero***	**Peripartum**	**Late**
n	20	15	16	13
Female (%)	9 (45)	9 (60)	6 (38)	3 (23)
Breastfed (%)	17 (85)	13 (87)	14 (88)	13 (100)
Median number of study visits (range) evaluated for CMV DNA	6 (4–6)	7 (3–10)	5.5 (3–9)	7 (3–10)
Mean HIV-1 viral load peak (SD)	NA	6.7 (0.86)	7.1 (0.48)	6.8 (0.71)
Mean set-point HIV-1 viral load (SD)[a]	NA	6.2 (0.68)	6.4 (0.61)	5.6 (1.0)
Deaths[b] (%)	3 (15)	8 (53)	8 (50)	4 (31)

CMV, cytomegalovirus; NA, not applicable; SD, standard deviation of the mean.

[a] Set-point HIV-1 viral loads were not defined for infants who died before their first postpeak viral load measurement; this included one infant in the in utero group, three infants in the peripartum group and three infants in the late group.

[b] Deaths during the first year of life for HIV-exposed uninfected infants, deaths during the first 2 years of life for the HIV-infected infants.

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Table 2

Cytomegalovirus detection in children grouped by mode of HIV-1 acquisition.

	Cumulative % CMV DNA detected			
Age	**HIV-uninfected[a], *n* = 20**	**HIV *in utero*, *n* = 15**	**HIV peripartum *n* = 16**	**HIV late, *n* = 13**
Birth[b]	6.3	29	0	0
1 month	20	53	13	23
3 months	90	93	75	85
6 months	90	93	81	92
9 months	90	100	81	92
12 months	95	100	88	92
15 months	_[c]	100	88	92
18 months	_[c]	100	88	92
21 months	_[c]	100	94	92
24 months	_[c]	100	94	92

[a] Infants with no HIV-1 detection exited the study at 12 months of age.

[b] Cord blood tested at birth.

[c] Not tested.

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Table 3

Detection of cytomegalovirus DNA in HIV-exposed infants following acute infection.

	Months after first CMV detection							
	1–3	**4–6**	**7–9**	**10–12**	**13–15**	**16–18**	**19–21**	**22–24**
HIV infected[a]								
DNA+/tested (%)	20/22 (91%)	15/21 (71%)	13/18 (72%)	6/12 (50%)	7/10 (70%)	4/9 (44%)	3/7 (43%)	0/1 (0%)
HIV uninfected[b]								
DNA+/tested (%)	13/18 (72%)	9/17 (53%)	8/17 (47%)	3/4 (75%)	NT	NT	NT	NT
P[c]	0.2	0.3	0.2	0.6				

NT, not tested.

[a] Excludes infants with late HIV-1 acquisition.

[b] HIV-uninfected infants exited the study at 12 months of age.

[c] *P* values were derived from Fisher's exact test.

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