MAJOR ARTICLE

Clinical and Virologic Manifestations of Primary Epstein-Barr Virus (EBV) Infection in Kenyan Infants Born to HIV-Infected Women

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(See the editorial commentary by Balfour and Verghese on pages 1787–9.)

Background. Human immunodeficiency virus (HIV) infection is a risk factor for Epstein-Barr virus (EBV)–associated lymphomas. Characterizing primary infection may elucidate risk factors for malignancy.

Methods. To describe clinical and virologic manifestations of primary EBV infection among infants born to HIV-infected women, specimens were utilized from a cohort study conducted in Nairobi, Kenya. HIV and EBV viral loads were measured serially in plasma. EBV serology was performed on EBV DNA–negative infants. Monthly clinical examinations were performed by pediatricians.

Results. The probability of EBV infection by 1 year of age was .78 (95% CI, .67–.88) in HIV-infected and .49 (95% CI, .35–.65) in HIV-uninfected infants ($P < .0001$). At 2 years, probability of EBV infection was .96 (95% CI, .89–.99) in HIV-infected infants. Peak EBV loads were higher in HIV-infected versus HIV-uninfected infants (median 2.6 vs 2.1 $\log_{10}$ copies/mL; $P < .0001$). The majority of HIV-infected infants had detectable EBV DNA for >3 months (79%). Primary EBV infection was associated with cough, fever, otitis media, pneumonia, hepatomegaly, splenomegaly, and hospitalization in HIV-infected infants; conjunctivitis and rhinorrhea in HIV-uninfected infants.

Conclusions. EBV infection occurs early in infants born to HIV-infected women. HIV infection was associated with more frequent and higher quantity EBV DNA detection.

Keywords. EBV; primary infection; HIV; pediatric; herpesviruses.

Epstein-Barr virus (EBV) infects >95% of the global population with prevalence varying by sociodemographics and region. Infection with EBV is uncommon before the age of 5 in European and American children, after which seroprevalence increases through adulthood [1, 2]. This epidemiology differs from sub–Saharan Africa where many children acquire EBV before the age of 3 [3–6]. EBV infects via the oropharyngeal route, and is transmitted primarily through saliva. Following symptomatic primary infection, EBV DNA can be detected in blood from most individuals, and is rapidly cleared in 1–3 weeks [7, 8]; but in saliva, virus may be detected for months to years [8, 9].

EBV is the cause of infectious mononucleosis in adolescents and adults [10], but the majority of primary infections in children are asymptomatic or mild [11]. Symptoms reported in children include pharyngitis, rash, fever, hepatomegaly, and splenomegaly [12, 13], but are infrequently a cause for hospitalization [14]. Although latent EBV infection is usually silent, EBV is a major contributor to malignancies worldwide [15], including lymphoma, nasopharyngeal carcinoma, and gastric cancer. In a large prospective study, the risk of developing Hodgkin lymphoma was found to peak at

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2.4 years following infectious mononucleosis [16]. EBV is also the most common cause of cancer among children in equatorial Africa, endemic Burkitt lymphoma [17, 18]. Peak Burkitt lymphoma incidence in African children occurs in childhood, highlighting the potential importance of primary infection in the risk of malignancy [19, 20]. EBV additionally causes non–Hodgkin lymphoma in the context of human immunodeficiency virus (HIV). HIV-infected children are at an estimated >40-fold increased risk for cancer [21] and many of these malignancies are associated with EBV. Risk factors for HIV-associated malignancy include a diagnosis of AIDS, low CD4 lymphocyte cell count, unsuppressed HIV replication, short antiretroviral therapy (ART) duration, and high EBV viral load [22–25].

We hypothesized that primary HIV infection may result in earlier EBV infection and poor control of EBV replication and dissemination, which could in turn elevate a child's long-term risk of developing cancer. In a cohort of HIV-infected and uninfected infants born to HIV-infected mothers, we describe the incidence and correlates of EBV infection, the kinetics of EBV viremia, and the clinical manifestations of primary EBV infection.

METHODS

Study Design

This longitudinal study utilized repository specimens and data from an HIV-1 transmission study in Nairobi, Kenya [26, 27]. The current study leveraged this historic observational cohort to compare EBV detection and viral loads between HIV-infected and -uninfected infants. Sample size calculations were based on an earlier paper reporting 68% EBV seroprevalence in Nigerian children screened at 6–16 months of age [5]. With a sample size of 50 HIV-infected and 50 HIV-uninfected infants, we estimated the study would be powered to detect a hazard ratio (HR) of ≥2.2 for the effect of HIV on EBV detection. We later modified our sample size to all eligible HIV-infected infants ($n = 75$) to increase statistical power further. For EBV viral load, we estimated 80% power to detect a minimum 0.6 $\log_{10}$ mean difference in peak EBV viral load between HIV-infected and -uninfected infants, assuming a standard deviation (SD) of 1.0 $\log_{10}$. Both power calculations assumed 2-sided tests with 80% power and $\alpha = 0.05$. All sample size calculations and statistical analyses were planned a priori, with the exception of the clinical manifestations, which were performed post hoc after observing the high incidence of early EBV infection in the cohort.

Inclusion criteria were survival and follow-up to ≥3 months and well-defined timing of HIV infection. Because maternal antibody is highly protective, we assumed few EBV infections would occur before 6 months of age. All eligible HIV-infected infants were included in the study. To meet the sample size of 50 HIV-uninfected infants, a random sample was drawn from the 338 HIV-uninfected infants satisfying inclusion criteria. This was done by assigning a uniformly distributed set of pseudo-random numbers between zero and 1 to each infant ID number, sorting by random number, and selecting the first 50 in rank.

Participants

The study was approved by the University of Washington Institutional Review Board and the Kenyatta National Hospital Ethics and Research Committee. Mothers provided written informed consent; recruitment, enrollment, and follow-up are detailed elsewhere [26–28]. At enrolment, women provided a detailed medical history and sociodemographic data. HIV-seropositive pregnant women were enrolled between 1999–2003 and provided with zidovudine from 32 weeks' gestation for the prevention of mother-to-child HIV transmission (PMTCT) [29]. The study was conducted before ART became widely available in Kenya; participants in this study received no ART other than PMTCT. HIV-infected infants were followed for 2 years, while HIV-uninfected infants exited the study at 12 months.

Clinical Assessments

As part of the historic cohort study, mothers and infants attended monthly clinic visits in which a study pediatrician collected information regarding infant illness, and conducted a physical examination [26, 27]. Infant symptoms were systematically assessed at each study visit with the aid of a standardized questionnaire, which included measurement of vital signs; examination of the head, chest, abdomen, and skin; and assessment for edema, dehydration, lymphadenopathy, and neurologic symptoms. Symptoms were recorded separately as maternally reported since previous visit, maternally reported at current visit, or physician-observed at current visit.

Specimen Collection and Infant HIV Diagnosis and Viral Load

Blood was collected at 32 weeks' gestation, delivery, 1 and 3 months postpartum, and every 3 months thereafter from mothers and infants. Infant HIV was diagnosed by polymerase chain reaction (PCR) detection of HIV gag DNA from dried blood spots [30] or HIV RNA viral load in plasma [31], whichever was detected first.

Measurement of EBV Viral Load

EBV was measured in the same plasma specimens used for HIV viral loads, using a real-time PCR assay. DNA was extracted from 200 µL of plasma specimens using QIAamp 96 DNA Blood Kit (Qiagen, Valencia, CA) and eluted into 100 µL Qiagen AE buffer. BALF5 primers (forward 5′-CGGAAGC CCTCTGGACTTC-3′; reverse 5′-CCCTGTTTATCCGATG GAATG-3′) and probe (FAM 5′-TGT ACA CGC ACG AGA AAT GCG CC-3′-TAMRA) were used to detect EBV genome in 10 µL of the extracted DNA [32]. Each 30 µL PCR reaction contained 15 μL of 2x Quantitect Multiplex PCR Master Mix (Qiagen), 833 nm primers, 100 nm probe, and internal control. The limit of detection for the assay was 50 EBV DNA copies/mL of plasma.

EBV Serology

We assumed that positive EBV DNA results represented true infections, and performed confirmatory serology on the final specimen available from infants who were EBV DNA negative. Because maternal antibody confounds interpretation of results before 6 months, we excluded infants whose last specimen was tested before 6 months (11 HIV-infected and 3 HIV-uninfected). Based on these criteria, 54 samples were selected for serology; 10 had no remaining specimen at their last visit, so we tested the closest available specimen collected at ≥6 months, and 8 infants had no remaining sample, for a final set of 46 infants.

Antibodies for viral capsid antigen (VCA)–immunoglobulin M (EBV VCA-IgM ELISA II kit, Wampole Laboratories, Princeton, NJ), VCA–immunoglobulin G (EBV VCA-IgG ELISA II kit, Wampole), and EBV nuclear antigen–1-IgG (ENBA-1 IgG ELISA II, Wampole) were measured from 200 μL plasma, according to the manufacturer's instructions. Results were reported as positive, equivocal, or negative; each infant was assigned a final EBV result of positive for the detection of ≥1 antibody, or negative for all 3.

Statistical Analyses

Stata SE v11.2 was used for statistical analyses (StataCorp, College Station, TX), and all *P* values are for 2-tailed tests. Kaplan–Meier survival analysis and the log-rank test were used to compare time to EBV infection between HIV-infected and -uninfected infants. EBV infection was defined as EBV DNA or antibody detection in plasma, and HIV-infection status was treated as a time-dependent covariate in the model. Comparisons of HIV-infected and -uninfected children were made on data censored at 12 months of follow-up to account for differential follow-up in the 2 groups. Similarly, Cox proportional hazard regression was used to estimate HR and 95% confidence intervals (95% CIs) for a priori-defined correlates of EBV infection during the first year of life; data were censored at 12 months or death, whichever occurred first. Kaplan–Meier survival analysis was used to estimate time to EBV suppression following first EBV detection in HIV-infected infants who had at least 1 or more study visits conducted at least 3 months after acute EBV infection.

Peak EBV viral loads were compared between groups using the Mann–Whitney *U* test. Peak EBV viral load was defined as the highest of all EBV positive measurements.

Generalized estimating equations with a binomial link function and robust standard errors were used to estimate odds ratios (ORs) for clinical symptoms during an acute EBV

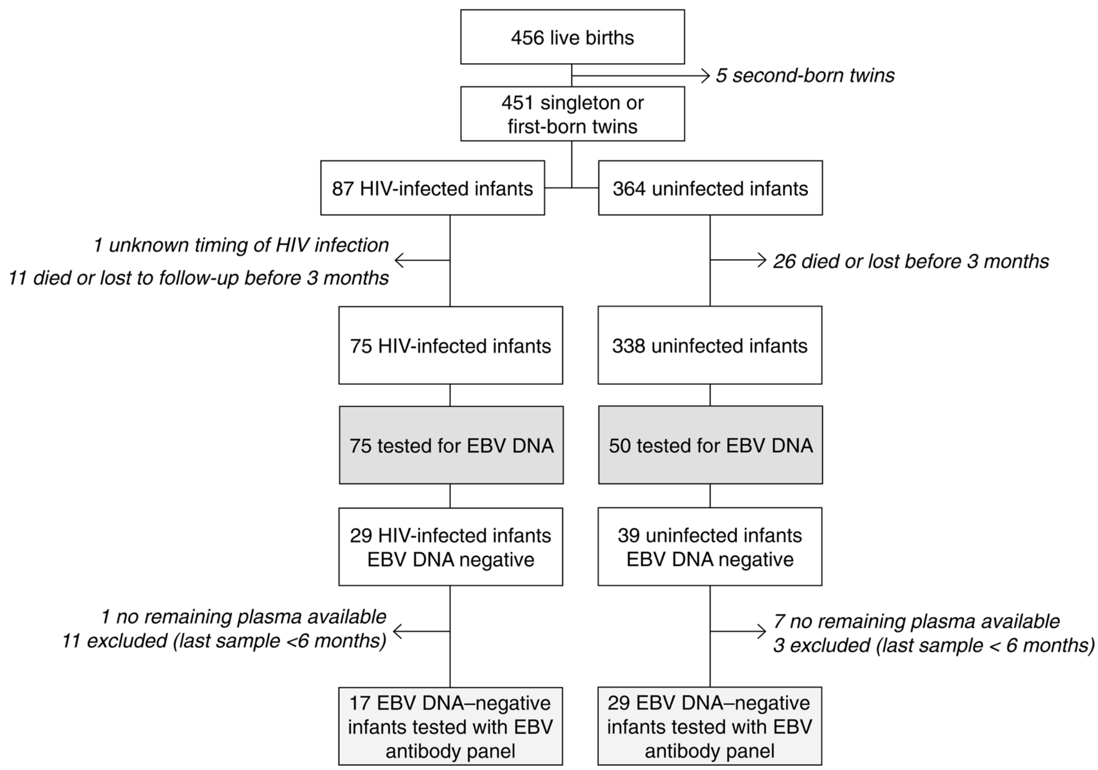

Figure 1. Diagram of study procedures.

Table 1. Characteristics of Study Participants

Characteristic	Median (IQR) or % (n/N) HIV-uninfected (N = 50)	HIV-infected (N = 75)	*P* Value
Follow-up[a]			
Months of follow-up	12 (10–12)	12 (6–24)	.1
Number of study visits	5 (2–9)	6 (3–10)	.2
Number of visits screened for EBV	4 (4–5)	5 (3–7)	.2
Number of clinical assessments	12 (10–13)	12 (7–16)	.5
Infant characteristics			
Female sex	44% (22/50)	43% (32/75)	>.9
Low birth weight[b]	2.1% (1/48)	6.7% (5/75)	.4
Premature[b]	2.3% (1/44)	3.1% (2/65)	>.9
Breastfed	64% (32/50)	89% (67/75)	.001
1 y mortality	2% (1/50)	29% (22/75)	<.001
2 y mortality	NA	51% (38/75)	NA
Infant HIV infection			
HIV first detected at 1 mo	NA	79% (59/75)	NA
HIV first detected >1 mo	NA	21% (16/75)	NA
Maternal characteristics			
Age at enrollment	25 (22–29)	25 (21–28)	.6
Primiparous	26% (13/50)	11% (8/75)	.03
Parity	1 (0–2)	2 (1–2)	.1
Years of education	9 (8–12)	8 (7–11)	.02
Maternal death	2% (1/50)	16% (12/75)	.009
Maternal immunologic status in pregnancy			
HIV viral load at 32 wk	4.6 (4.2–5.2)	5.1 (4.7–5.5)	.001
CD4% at 32 wk	24 (19–29)	19 (14–26)	.006
CD4 cells/mm^3 at 32 wk	453 (288–596)	370 (237–565)	.1

Abbreviations: EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; IQR, interquartile range; NA, not applicable.

[a] HIV-uninfected infants exited the study at 12 months per protocol; HIV-infected infants were followed for an additional year.

[b] Among infants assessed <24 hours of birth, low birth weight was defined as <2.5 kg, premature as <37 weeks by Dubowitz score; children born at home (n = 3) and at another health care facility (n = 9) did not have gestational age assessment; and 2 infants born at the study site had missing Dubowitz scores.

infection visit versus EBV-negative visits prior to EBV detection. Acute EBV infection visits were defined as all visits following the last EBV DNA–negative visit and up to and including the first EBV DNA–positive visit, provided the last negative and first positive were no more than 3 months apart. EBV-negative visits were defined as all visits up to and including the last EBV-negative test prior to EBV infection. Because EBV PCRs were performed at less-frequent intervals than monthly clinical assessments, the appearance of a symptom between 2 EBV testing intervals was counted toward the next EBV test visit. Because infants with HIV acquisition after 1 month of age (n = 16) would have had both HIV-negative and HIV-positive visits assessed prior to EBV infection, these infants were excluded from the clinical analysis.

RESULTS

Patient Information

Figure 1 illustrates the selection of infants for EBV studies. A total of 565 EBV viral load measurements were conducted on 75 HIV-infected infants and 50 HIV-uninfected infants (Table 1). HIV-infected infants were followed for a median of 12 months and were tested for EBV at a median of 5 visits. HIV-uninfected infants were followed for a median of 12 months and were tested at a median of 4 visits. The majority of HIV-infected infants acquired HIV before 1 month of age (79%), and more than half of the HIV-infected infants died during the first 2 years of life. To ensure that the 50 HIV-uninfected infants were representative of the whole HIV-unexposed cohort, we compared the parameters listed in Table 1 between selected and unselected infants and found no significant differences (data not shown).

EBV Acquisition

Overall, 73% (55/75) of HIV-infected and 40% (20/50) of -uninfected infants had either detectable EBV DNA or antibody. EBV DNA was detected in 46/75 (61%) HIV-infected and 11/50 (22%) HIV-uninfected infants. Eighteen (9 HIV-infected and 9 HIV-uninfected) infants were EBV DNA negative and EBV seropositive. EBV DNA was detected in plasma throughout the first 2 years of life, was detected as early as 1 month of age (1 HIV-infected infant), and was commonly detected before 6 months of age (Figure 2*A*). The probability of EBV infection at 12 months was 0.49 (95% CI, .35–.65) in HIV-uninfected and 0.78 (95% CI, .67–.88) in HIV-infected infants. Censoring at 12 months, the mean time to first EBV detection was 9.0 months in HIV-infected (95% CI, 8.2–9.8) compared to 11 months in HIV-uninfected (95% CI, 11–12, log-rank $P < .0001$) infants. At 24 months of age, the probability of EBV detection was 0.96 (95% CI, .89–.99) in HIV-infected children.

EBV Viral Loads

EBV viral loads were detected in the range of 1.8–5.2 $\log_{10}$ DNA copies/mL. EBV viral loads were higher in HIV-infected compared to HIV-uninfected infants (Figure 2*B* and *C*); the median peak EBV viral load was 2.6 $\log_{10}$ DNA copies/mL (interquartile range [IQR] = 2.4–3.0) in HIV-infected compared to 2.1 $\log_{10}$ DNA copies/mL (IQR = 1.9–2.4; $P < .0001$) in HIV-uninfected infants.

EBV Containment

Among the 33 HIV/EBV coinfected, EBV viremic infants with follow-up data ≥3 months after EBV detection, 11 became EBV undetectable at a later visit (33%), at a mean of 15 months (95% CI, 12–18) (Figure 2*D*). As a sensitivity analysis for transiently negative and/or false-negative assays, we evaluated EBV suppression employing a more stringent definition, the first of 2 consecutive negative tests. There were 24 HIV/EBV-

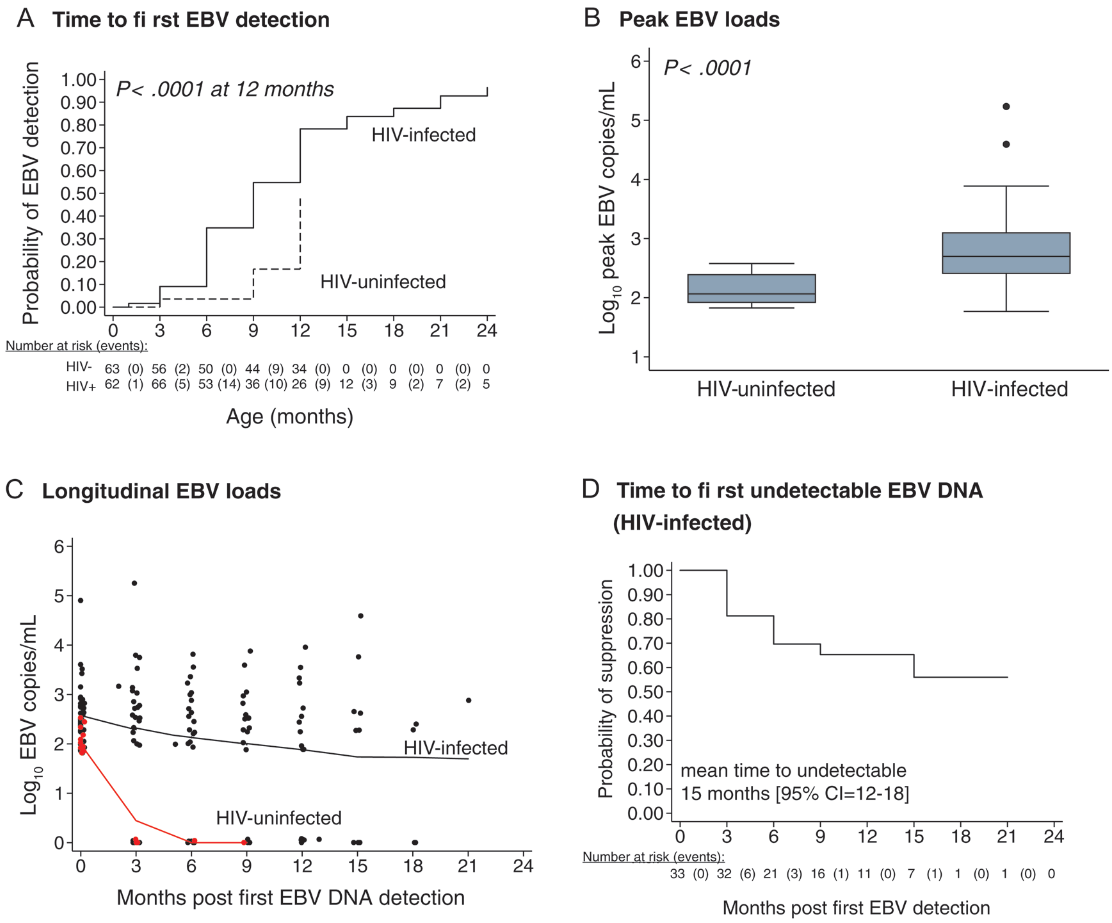

Figure 2. EBV acquisition, viral loads, and containment in HIV-infected and -uninfected Kenyan infants. *A*, Probability of EBV infection, with time-updated HIV-1 status; *P* value for log-rank test compares time to EBV with censoring at 1 year. *B*, Peak EBV viral loads (medians and IQRs). *C*, Lowess curves showing EBV viral load post EBV detection. *D*, Probability of EBV suppression among the 33 HIV-infected infants with ≥1 visit after first detection.

coinfected infants with ≥2 visits after EBV detection, and 6 of these infants (25%) achieved 2 consecutive negative EBV tests with a mean time to EBV suppression of 17 months (95% CI, 14–20, data not shown).

EBV-infected infants were categorized as "good controllers" (DNA never detected, or detected once) or "poor controllers" (EBV DNA detected at ≥3 months after first detection). Thirteen HIV-infected and 8 uninfected infants had EBV DNA detected only at their last study visit. Of the 42 EBV/HIV-coinfected infants with follow-up visits, 62% were classified as poor controllers and 38% as good controllers, whereas all 12 HIV-uninfected infants with follow-up testing were classified as good controllers ($P < .001$).

Correlates of EBV Infection

We examined sociodemographic, maternal, and infant correlates of EBV detection in the first year of life (Table 2). In the unstratified analysis, maternal prenatal CD4 < 20% (HR = 1.6; 95% CI, 1.0–2.6), maternal HIV viral load >4.5 $\log_{10}$ RNA copies/mL (HR = 2.0; 95% CI, 1.1–3.8), and infant HIV infection (HR = 2.5; 95% CI, 1.5–4.3) were associated with an increased rate of EBV acquisition. We found no significant associations when stratifying by infant HIV infection.

Clinical Manifestations of Acute EBV Viremia

Clinical symptoms were compared between acute EBV infection visits and EBV-negative visits prior to infection separately for HIV-infected and HIV-uninfected infants (Table 3). Lymphadenopathy was commonly observed, but was not associated with EBV detection in either HIV-infected (OR = 1.6; 95% CI, .70–3.7; $P = .3$) or HIV-uninfected (OR = 3.1; 95% CI, .51–18; $P = .2$) infants (data not shown). In HIV-infected infants, cough (OR = 3.8; 95% CI, 1.7–8.3), fever (maternal report OR = 2.7; 95% CI, 1.2–5.8 and measured at visit OR = 2.7; 95% CI, 1.1–6.7), otitis media (OR = 3.6; 95% CI, 1.3–9.5), pneumonia (OR = 2.3; 95% CI, 1.1–4.8), splenomegaly (OR = 4.4; 95% CI, 1.9–10), and hepatomegaly (OR = 2.8; 95% CI, 1.0–7.5) were more commonly recorded at the acute

Table 2. Correlates of EBV Infection During the First Year of Life

Characteristic	Hazard ratio (95% CI)		
	All infants (N = 125)	HIV-uninfected infants (N = 50)	HIV-infected infants[a] (N = 75)
Maternal and sociodemographic			
Maternal age at enrollment	0.99 [.93–1.0]	1.0 [.89–1.1]	0.99 [.93–1.1]
Years of education	0.98 [.90–1.1]	0.92 [.78–1.1]	1.0 [.94–1.1]
Primiparous	1.2 [.68–2.3]	1.5 [.61–3.8]	1.9 [.77–4.4]
Parity	0.89 [.74–1.1]	0.84 [.57–1.2]	0.83 [.66–1.0]
HIV viral load >4.5 $\log_{10}$ copies/mL	**2.0 [1.1–3.8]**[b]	1.5 [.57–3.7]	1.9 [.75–4.8]
CD4 < 20%	**1.6 [1.0–2.6]**[c]	1.5 [.61–3.9]	1.4 [.75–2.4]
CD4 < 350 cells/mm^3	1.1 [.67–1.8]	1.1 [.47–2.8]	0.99 [.55–1.8]
Infant baseline characteristics			
Female sex	0.89 [.54–1.5]	0.77 [.31–1.9]	1.0 [.55–1.8]
Ever breastfed	1.0 [.58–1.8]	0.78 [.32–1.9]	0.56 [.24–1.3]
Birth weight	0.78 [.46–1.3]	0.80 [.35–1.8]	0.76 [.36–1.6]
HIV infection	**2.5 [1.5–4.3]**[d]	NA	NA

All estimates are unadjusted. Maternal HIV viral load and CD4 measurements were at 32 weeks' gestation. Covariates with significance at $P \leq .05$ are shown in bold.

Abbreviations: CI, confidence interval; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; NA, not applicable.

[a] No infants acquired EBV prior to HIV-1 infection.

[b] $P = .03$.

[c] $P = .05$.

[d] $P = .001$.

EBV infection visit compared to EBV-negative visits. HIV-infected infants were also more than twice as likely to be hospitalized during acute EBV infection (OR = 2.7; 95% CI, 1.0–7.2).

In HIV-uninfected infants, acute EBV infection visits were significantly associated with conjunctivitis (OR = 5.4; 95% CI, .99–29) and rhinorrhea (OR = 4.4; 95% CI, 1.0–19), and there were trends for association with splenomegaly, hepatomegaly, and maternal-reported fever.

Because we were unable to define an acute EBV infection visit for the 18 infants who were EBV DNA negative/seropositive, we performed a sensitivity analysis excluding these; all clinical manifestations were similar with the exception that the diagnosis of an upper-respiratory-tract infection was more commonly reported at acute EBV infection visits in HIV-infected infants (OR = 2.6; 95% CI, 1.1–5.9; $P = .03$). This association was not statistically significant in HIV-uninfected infants (OR = 2.2; 95% CI, .40–12; $P = .4$).

DISCUSSION

In this study of HIV-exposed Kenyan infants, we found a high incidence of EBV infection during the first year of life. HIV-infected infants had earlier and more frequent EBV acquisition, and higher EBV viral loads compared to HIV-uninfected infants. Among HIV-infected infants, containment of EBV was variable, with some infants never having detectable DNA viremia and others persistently viremic. Finally, we found that acute EBV infection manifested clinically in HIV-infected infants, and distinctly from HIV-uninfected infants.

The incidence of EBV detection in our study is consistent with earlier estimates of HIV-uninfected children in East Africa [4–6], and in North American HIV-1 cohorts, which reported a 55%–65% incidence of EBV in HIV-uninfected and 65%–73% at 2 years in HIV-infected infants [9, 13]. Socioeconomic indicators and crowding have previously been identified as correlates of EBV acquisition [3, 33, 34]. In our study, maternal HIV viral load and immunosuppression were strongly associated with infant EBV detection. EBV shedding in saliva is elevated during immunosuppression [9, 35, 36], and this could explain the association between maternal immune status and infant EBV detection. Additionally, maternal immunosuppression may impair placental transfer of EBV-specific antibodies to the infant, as our study team has previously demonstrated with measles-specific IgG [37].

To date, few studies have examined the impact of infant HIV on primary EBV infection [9, 13, 38, 39]. We found that infant HIV infection was associated with increased EBV infection, an observation that could be due to both maternal and/or infant factors. Indeed, the high incidence of EBV detection prior to 6 months of age in HIV-infected infants suggests maternal antibody is not a substantial barrier to infant EBV infection. Additionally, generalized dysregulation of both B- and T-cell immune responses may contribute to the earlier acquisition of EBV in this group.

Infant HIV was also associated with higher EBV viral loads. This finding is consistent with 2 previous natural history studies conducted in children reporting more frequent detection of EBV DNA in blood [38] and saliva [9] in HIV-infected children. The identification of EBV DNA–negative EBV-seropositive infants, and the rapid disappearance of EBV DNA in the 3 HIV-uninfected infants suggest systemic EBV viremia is of short duration. In the setting of infectious mononucleosis (IM), EBV becomes undetectable in 1–3 weeks [7, 8]. In contrast, HIV-infected infants in our study were generally poor controllers of EBV viremia; the average time to suppress virus to undetectable levels was >1 year. However, a small subset of HIV-infected infants exhibited relatively better EBV containment. The dichotomy of these 2 patterns of primary EBV infection raises the intriguing possibility that these 2 groups may have different risks of developing malignancy in the future. Nakai and colleagues have described a similar phenotype in 1–16 year olds with IM; some children reduced virus quickly ("rapid regression") and others more slowly ("slow regression") [40]. Defining the genetic, immunologic, and viral factors

Table 3. **Clinical Manifestations of Primary EBV Infection**

	HIV-uninfected			HIV-infected (early)[a]		
	Visits with symptom			Visits with symptom		
	EBV-negative visits, % (N = 113)	Acute EBV visits, % (N = 11)	OR (95% CI)	EBV-negative visits, % (N = 169)	Acute EBV visits, % (N = 36)	OR (95% CI)
Maternal report to be present at current clinic visit						
Cough	70	82	1.9 [.37–9.4]	47	81	3.8 [1.7–8.3]
			$P = .5$			$P = .001$
Fever	33	55	2.8 [.88–8.9]	31	61	2.7 [1.2–5.8]
			$P = .08$			$P = .02$
Maternal report to have occurred since last clinic visit						
Hospitalized	4.4	0	. . .[b]	6.6	19	2.7 [1.0–7.2]
						$P = .04$
Diagnosed by pediatrician at current clinic visit						
Fever	16	27	1.8 [.44–7.2]	17	39	2.7 [1.1–6.7]
			$P = .4$			$P = .03$
Conjunctivitis	6.2	27	5.4 [.99–29]	7.7	8.3	1.1 [.28–4.5]
			$P = .05$			$P = .9$
Rhinorrhea	46	82	4.4 [1.0–19]	27	39	1.3 [.57–2.8]
			$P = .05$			$P = .6$
Pneumonia	4.4	0	. . .[b]	18	33	2.3 [1.1–4.8]
						$P = .03$
Otitis media	8.0	18	1.4 [.19–9.9]	5.9	22	3.6 [1.3–9.5]
			$P = .7$			$P = .01$
Splenomegaly	1.8	9.1	5.5 [.57–54]	8.3	31	4.4 [1.9–10]
			$P = .1$			$P = .001$
Hepatomegaly	0.88	9.1	7.2 [.53–100]	8.3	22	2.8 [1.0–7.5]
			$P = .1$			$P = .05$

Only symptoms with significance at $P \leq .05$ in at least 1 stratum are shown.

Abbreviations: EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; OR, odds ratio.

[a] N = 59 infants with HIV detection by 1 month of age.

[b] Insufficient number of cases in group to estimate OR.

discriminating good from poor EBV controllers may have high relevance for both the development of an efficacious EBV vaccine and for understanding the early risk factors for EBV-associated malignancies.

Our findings are also consistent with a recent study published by Piriou and colleagues, who demonstrated that healthy children residing in malaria-holoendemic region with high incidence of endemic Burkitt lymphoma had both earlier acquisition of EBV and higher EBV viral loads than children in a region with seasonal malaria and low endemic Burkitt lymphoma incidence [6]. We also found a high incidence of EBV infections prior to 6 months of age, particularly among the HIV-infected infants. Piriou speculated that earlier EBV acquisition may be a risk factor for poor EBV containment, and subsequent Burkitt lymphoma. Together with the Piriou study, our data lend additional support to a unifying hypothesis that early acquisition of EBV and poorly controlled primary infection may be important cofactors for future risk of EBV-related malignancies. On a population level, it is also notable that only a minority of infants with Burkitt lymphoma are HIV infected. This finding is somewhat at odds with our data demonstrating that HIV infection is associated with poorer control of EBV replication, but may be attributable to the fact that untreated HIV-infected infants in sub–Saharan Africa have a phenomenally high mortality rate, and many of those most at risk for development of Burkitt lymphoma may not have lived long enough to develop malignancy. As treatment coverage of HIV-infected infants improves regionally, it will be important to watch trends in lymphoma incidence closely.

Finally, we found that primary EBV infection may manifest differently in HIV-infected and HIV-uninfected infants, and cause substantial morbidity in HIV-infected infants. In HIV-uninfected infants, acute EBV infection was accompanied by mild, transient mucosal manifestations (conjunctivitis and rhinorrhea). In contrast, HIV-infected infants had a more severe spectrum of clinical symptoms during acute EBV infection, including splenomegaly, hepatomegaly, and pneumonia, and were more than twice as likely to be hospitalized. These findings suggest that viremic episodes of acute EBV infection may be an important cause of morbidity during acute infant HIV-1 infection, and warrants further study. Cervical lymphadenopathy, though ubiquitous in infectious mononucleosis, was not associated with acute EBV in this cohort, in either HIV-uninfected or HIV-infected infants. Concurrent primary HIV infection, and a high rate of non-EBV-related infectious disease in both groups undoubtedly masked our ability to detect less specific symptoms of acute EBV, and may also have affected our sensitivity to detect symptoms differentially in HIV-infected and HIV-uninfected strata.

Our study has many important strengths, including longitudinal assessment and highly detailed clinical examinations conducted by pediatricians. Weaknesses include the short 1-year follow-up period in HIV-uninfected infants, which limits our ability to describe their later EBV acquisition. Viral dynamics may vary by biologic compartment, and we may have observed different patterns of detection and persistence if we evaluated other tissues, such as saliva or whole blood. Additionally, the HIV-uninfected infants in our study may not be representative of HIV-*unexposed* infants; HIV-exposed uninfected infants have elevated morbidity and mortality, and altered immunologic profiles compared to unexposed infants (reviewed in [41]). Because we performed serology only at the last visit of EBV DNA-negative infants, we likely overestimate age at EBV infection, and underestimate hazard ratios in our correlates analysis. Finally, in our clinical analysis, we were unable to identify an acute infection visit for infants who were EBV DNA negative but EBV seropositive; however, our sensitivity analysis indicated that exclusion of these cases did not significantly alter our findings.

In summary, EBV was acquired early in this cohort, and its acquisition was altered by infant and/or maternal HIV infection. During the first 2 years of life, most HIV-infected infants had poorly suppressed EBV viremia, but a subset contained EBV rapidly. The long-term relevance of good versus poor containment of primary EBV infection, and the impact of ART will be important areas of study as pediatric ART coverage further improves in this region, and the population at risk for EBV-associated malignancies increases.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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