

# Reduced Mother-to-Child Transmission of HIV Associated with Infant but not Maternal GB Virus C Infection

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(See the editorial commentary by Björkman and Widell, on pages 1358–60.)

**Background.** Prolonged coinfection with GB virus C (GBV-C) has been associated with improved survival in human immunodeficiency virus (HIV)–infected adults. We investigated whether maternal or infant GBV-C infection was associated with mother-to-child transmission (MTCT) of HIV-1 infection.

**Methods.** The study population included 1364 HIV-infected pregnant women enrolled in 3 studies of MTCT of HIV in Bangkok, Thailand (the studies were conducted from 1992–1994, 1996–1997, and 1999–2004, respectively). We tested plasma collected from pregnant women at delivery for GBV-C RNA, GBV-C antibody, and GBV-C viral genotype. If GBV-C RNA was detected in the maternal samples, the 4- or 6-month infant sample was tested for GBV-C RNA. The rates of MTCT of HIV among GBV-C–infected women and infants were compared with the rates among women and infants without GBV-C infection.

**Results.** The prevalence of GBV-C RNA in maternal samples was 19%. Of 245 women who were GBV-C RNA positive, 101 (41%) transmitted GBV-C to their infants. Of 101 infants who were GBV-C RNA positive, 2 (2%) were infected with HIV, compared with 162 (13%) of 1232 infants who were GBV-C RNA negative (odds ratio [OR] adjusted for study, 0.13 [95% confidence interval {CI}, 0.03–0.54]). This association remained after adjustment for maternal HIV viral load, receipt of antiretroviral prophylaxis, CD4<sup>+</sup> count, and other covariates. MTCT of HIV was not associated with the presence of GBV-C RNA (adjusted OR [aOR], 0.94 [95% CI, 0.62–1.42]) or GBV-C antibody (aOR, 0.90 [95% CI, 0.54–1.50]) in maternal samples.

**Conclusions.** Reduced MTCT of HIV was significantly associated with infant acquisition of GBV-C but not with maternal GBV-C infection. The mechanism for this association remains unknown.

GB virus C (GBV-C), a flavivirus closely related to hepatitis C virus and not known to cause any disease, may have an inhibitory effect on HIV-1 replication. GBV-C viremia often persists for years, but it is eventually cleared in 50%–75% of people, at which time antibodies to the GBV-C E2 envelope usually appear [1]. Persistent coinfection with GBV-C virus was associated with im-

proved survival rates among HIV-infected adults in 2 of 3 recently reported longitudinal cohort studies [2–4], with the effect depending on the time chosen for CD4<sup>+</sup> cell count adjustment. Loss of GBV-C RNA in previously infected individuals was associated with worse survival rates, compared with those who were never in-

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fects. Some investigators believe that GBV-C infection may improve survival rates over the long term [3, 5], due to several biologically plausible mechanisms including interference with HIV viral replication [6], increased production of chemokine ligands for the CC chemokine receptor 5 (CCR5) [7], downregulation of the CCR5 receptor [7, 8], and modulation of the T-helper cytokines [9]. However, other researchers suggest that the causal relationship may be reversed and that GBV-C infection status is, rather, a phenomenon secondary to HIV progression [2, 4]. The nature of the interaction between GBV-C and HIV remains unclear.

If GBV-C inhibits HIV replication, we hypothesized that maternal or infant infection with GBV-C might also reduce mother-to-child-transmission (MTCT) of HIV. Worldwide, approximately 700,000 children are infected with HIV annually, largely through MTCT of HIV. Biologic mechanisms that inhibit HIV replication may be relevant to the prevention of this type of transmission. We conducted a study to test this hypothesis using data and stored blood collected during 3 large perinatal studies in Bangkok.

## METHODS

**Study population and design.** The study population consisted of participants in 3 perinatal HIV transmission studies in which women did not breast-feed (hereafter, “Peri-1,” “Peri-2,” and “Peri-3”) conducted jointly by the Thailand Ministry of Public Health and the US Centers for Disease Control and Prevention (CDC) at 2 large Bangkok hospitals between 1992 and 2004 [10–12]. Peri-1 (1992–1994) was a natural history study conducted prior to the use of antiretroviral prophylaxis for the prevention of MTCT of HIV. Peri-2 (1996–98) was a randomized, placebo-controlled trial assessing the efficacy of short-course zidovudine prophylaxis. Women in the zidovudine group received 300 mg of zidovudine orally twice daily from 36 weeks gestation, once at onset of labor, and then every 3 h until delivery. Peri-3 (1999–2004) was an observational study to evaluate the implementation of the “Bangkok regimen” as described above, but with 4 weeks of zidovudine therapy added for neonates. Approximately 65% of mothers received both the antenatal and the delivery components of the regimen, 17% received a partial regimen, and 17% received no antiretroviral medications. All infants received zidovudine. A substudy population of 220 women who received the full regimen also received 200 mg of nevirapine orally once during labor, and their infants received 1 dose of nevirapine in addition to zidovudine.

HIV-infected women from these studies who had a blood specimen obtained at delivery that was available for testing as of March 31, 2004, and whose infant had a known HIV test result were included in the present study. Maternal plasma specimens collected at delivery were tested for GBV-C RNA, GBV-C antibody, and GBV-C viral genotype. Infants of GBV-C viremic

mothers who had a 4- or 6-month infant plasma specimen—the time during which GBV-C RNA has been detectable in GBV-C–infected infants in other studies [13, 14]—were tested for GBV-C RNA. Infants of mothers who tested negative for GBV-C RNA were assumed to be GBV-C RNA negative.

This study was approved by the Research Ethics Board, University of Toronto, Canada; the Institutional Review Board, CDC, Atlanta; and the Ethical Review Committees for Research in Human Subjects at the Thailand Ministry of Public Health and Siriraj Hospital, Bangkok, Thailand.

**Specimen collection and transport.** In the original studies, specimens were collected and tested for HIV, HIV viral load, CD4<sup>+</sup> count, and NK cell percentage, as described elsewhere [10, 11]. Frozen specimens were shipped on dry ice from Bangkok to Atlanta for GBV-C testing. Laboratory staff were blinded to subject data.

**GBV-C testing.** GBV-C viral RNA was extracted from 200  $\mu$ L of plasma by use of the QIAamp MinElute Virus Vacuum kit (Qiagen) and quantified with the Quantitect Probe RT-PCR kit (Qiagen), in accordance with the manufacturer’s instructions and as described elsewhere [15]. The lower limit of reliable detection of this quantitative reverse transcription polymerase chain reaction (RT-PCR) test is 1000 viral copies per reaction. Samples positive for GBV-C viral RNA, including a small number with 1–999 viral copies, were confirmed by genotype analysis (see below). Plasma specimens were tested for antibody to the GBV-C E2 envelope protein (anti-E2) by a  $\mu$ PLATE Anti-HGenv microtiter assay (Roche Diagnostics). To determine genotype, samples positive for GBV-C RNA were amplified by nested RT-PCR using genotype-specific primers [16] and resolved by polyacrylamide gel electrophoresis on 20% Tris-borate-EDTA gels (Invitrogen). To distinguish genotypes with a similar amplicon molecular size, restriction fragment length polymorphism analysis was carried out [17].

**GBV-C infection status.** We classified women into 1 of the following 4 mutually exclusive categories: (1) GBV-C RNA positive (with or without antibody), defined as  $\geq$ 1000 viral copies detected with RT-PCR reaction or 1–999 viral copies detected that we were able to genotype; (2) GBV-C antibody positive, defined as anti-E2 positive serology test result and GBV-C RNA negative; (3) GBV-C negative, defined as GBV-C RNA negative and anti-E2 negative serology test result; and (4) GBV-C RNA indeterminate, defined as 1–999 copies of virus detected that we were unable to genotype.

Infants were classified into 1 of the following 3 mutually exclusive categories: (1) GBV-C RNA positive, defined as  $\geq$ 1000 viral copies detected; (2) GBV-C RNA negative, defined as no virus detected or infant of a mother who tested negative for GBV-C RNA; and (3) GBV-C RNA indeterminate, defined as 1–999 copies of virus detected. Subjects (both mothers and infants) who were classified as GBV-C RNA indeterminate were excluded from their respective univariate analyses. However, as

maternal GBV-C status was not included in the final model, mothers with indeterminate GBV-C status were not excluded from the multivariate analysis of infant HIV infection.

**Statistical methods.** The statistical significance of the associations between maternal and infant GBV-C infection and MTCT of HIV was assessed with 2-sided *P* values by use of  $\chi^2$  or Fisher exact tests. Odds ratios (ORs) adjusted for participation in the Peri-1, Peri-2, or Peri-3 study were calculated by use of the Cochran-Mantel-Haenszel procedure. Logistic regression was also used to calculate odds ratios adjusted for multiple covariates and 95% Wald confidence intervals (CIs). Homogeneity of the odds ratios across studies was assessed with the Breslow-Day test. The 3 studies were combined for the multivariate analysis, and a variable indicating the perinatal study was included to adjust for any unmeasured confounding between studies. Variables associated with MTCT of HIV in univariate analysis that had a *P* value < .20 were included in the initial multivariate analysis. Manual modeling strategies were employed to control for confounding. HIV and GBV-C viral load were transformed to their  $\log_{10}$  value. If a linear relationship was not observed, continuous variables were categorized at generally used cut points or at levels defined by the relationship observed with MTCT of HIV. All analyses were performed with SAS (version 8.2; SAS Institute) or StatXact (version 6.0; Cytel Software).

## RESULTS

Table 1 shows selected characteristics of the 1364 HIV-infected pregnant women included in our study. The median number of lifetime sexual partners was 2 (interquartile range [IQR], 1–2), 9% were commercial sex workers, 2% were injection drug users (IDUs), and 13% had partners who were IDUs. Over the time period covered by the 3 studies, the median maternal age increased from 22 to 26 years (*P* < .001), the proportion of women with an educational level higher than primary increased from 34% to 44% (*P* = .01), and the proportion of women with partners who were IDUs increased from 7% to 16% (*P* < .001).

The 1364 women were classified as follows: 262 women (19%) were GBV-C RNA positive, including 12 women who tested positive for both virus and antibody; 176 (13%) were GBV-C antibody positive; 877 (64%) were GBV-C negative; and 49 (4%) were GBV-C RNA indeterminate (figure 1). Overall, 438 (32%) women had evidence of current or past GBV-C infection, as indicated by presence of GBV-C RNA or GBV-C antibody, respectively. Of 245 infants with definitive GBV-C results born to mothers who were GBV-C RNA positive, 101 (41%) acquired GBV-C RNA from their mothers (table 1). Women enrolled in Peri-1 appeared to have a higher prevalence of GBV-C RNA positivity, despite lower GBV-C viral loads, and were less likely to transmit GBV-C to their infants than women enrolled in Peri-2 or Peri-3. Prevalence of GBV-C genotype 2A declined

over the course of the studies, whereas genotypes 3 and 4 became more prevalent.

We observed a statistically significant association between infant GBV-C infection and reduced MTCT of HIV (*P* < .001) (table 2). In Peri-1, infant GBV-C RNA appeared highly protective against HIV acquisition; 0 of 21 infants with GBV-C RNA acquired HIV, compared with 62 (25%) of 248 infants not infected with GBV-C (OR, 0.00 [95% CI, 0.00–0.59]). A similar relationship was also observed in Peri-2, in which 1 (3%) of 29 infants infected with GBV-C acquired HIV, compared with 53 (15%) of 349 infants not infected with GBV-C (OR, 0.20 [95% CI, 0.03–1.50]), and in Peri-3, in which 1 (2%) of 51 infants infected with GBV-C acquired HIV, compared with 47 (7%) of 635 infants not infected with GBV-C (OR, 0.25 [95% CI, 0.03–1.85]). When data from all of the studies were combined, the association was highly significant, with a greater than 7-fold reduction in the odds of infant HIV acquisition (aOR adjusted for study, 0.13 [95% CI, 0.03–0.54]) among infants infected with GBV-C, compared with infants not infected with GBV-C.

We did not observe an association between the presence of GBV-C RNA in maternal samples and MTCT of HIV in any of the 3 studies or when the studies were combined (aOR adjusted for study, 0.94 [95% CI, 0.62–1.42]) (table 3). Among mothers who tested positive for GBV-C RNA, GBV-C genotype and maternal GBV-C viral load were also not associated with MTCT of HIV. The presence of maternal GBV-C antibody was found to be protective (*P* = .04) against MTCT of HIV only in Peri-1; this effect was not observed in Peri-2 or Peri-3, or when all studies were combined (aOR adjusted for study, 0.90 [95% CI, 0.54–1.50]). Among women who received antiretroviral therapy (ART), GBV-C RNA appeared somewhat more protective against MTCT of HIV (OR, 0.67 [95% CI, 0.28–1.61]), compared with women who did not receive ART (OR, 1.22 [95% CI, 0.77–1.92]), but not significantly so (*P* = 0.24, for interaction). Among coinfecting women in our study, HIV and GBV-C viral loads were significantly inversely correlated (Spearman  $\rho$ , –0.21; *P* < .001). However, when stratified by receipt of ART, viral loads appeared to be correlated only among women who received ART (Spearman  $\rho$ , –0.18; *P* = .05) and not among women who did not (Spearman  $\rho$ , –0.05; *P* = .56).

In multivariate analysis (table 4), the odds ratio for the association between infant GBV-C and MTCT of HIV remained essentially unchanged after adjustment for other covariates (aOR, 0.14), including maternal receipt of ART and maternal HIV viral load at delivery. Maternal CD4<sup>+</sup> count did not confound the association between infant GBV-C infection and MTCT of HIV and was not included in the final model. All analyses were initially adjusted for the perinatal study in which subjects originally participated, but, because receipt of ART was highly correlated with study, receipt of ART was substituted for the study variable in the final model, and this did not substantially change any odds ratio estimates. Although maternal HIV viral load is on the

**Table 1. Clinical and demographic characteristics of 1364 HIV-infected women and their infants in Bangkok Thailand, according to the perinatal study in which they originally participated.**

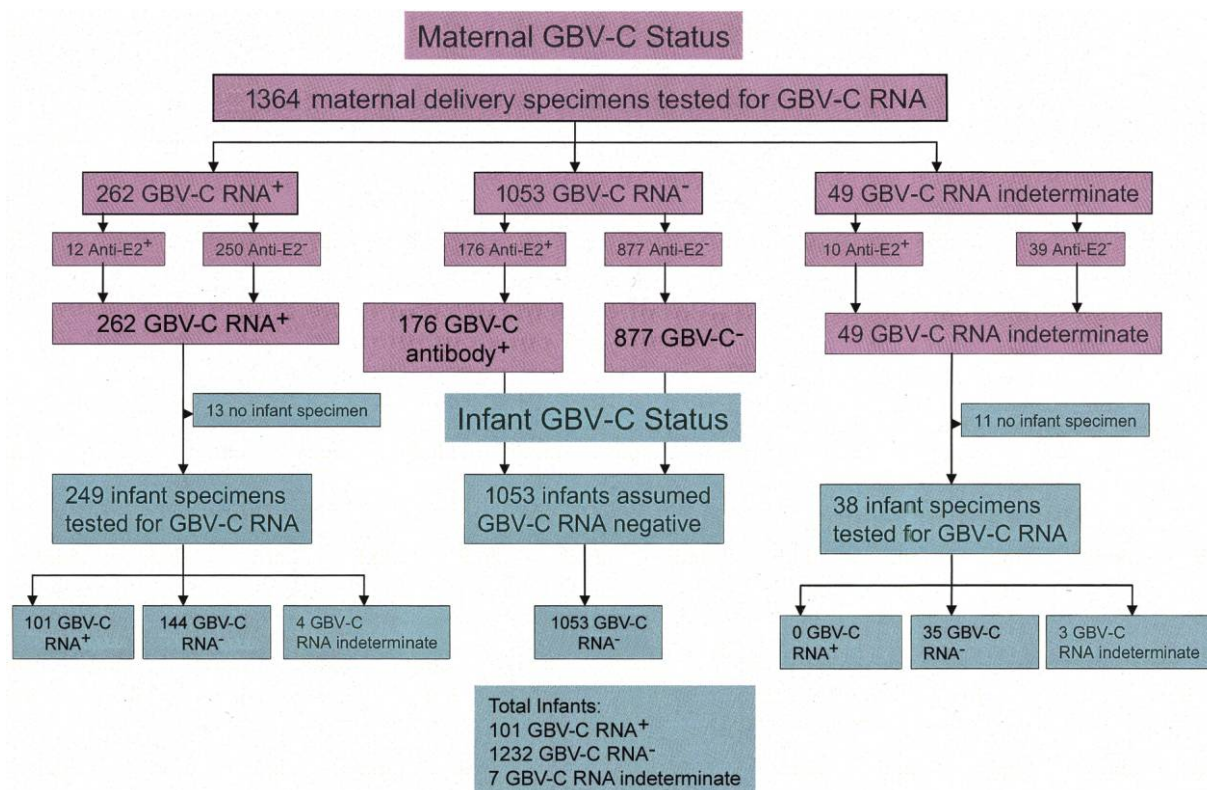
Variable	Original study			Overall
	Peri-1, 1992–1994	Peri-2, 1996–1997	Peri-3, 1999–2004	
<b>Maternal characteristic</b>				
GBV-C status				
RNA <sup>a</sup>	88/279 (32)	58/383 (15)	116/702 (17)	262/1364 (19)
Antibody <sup>a</sup>	31/279 (11)	46/383 (12)	99/702 (14)	176/1364 (13)
Negative	149/279 (53)	276/383 (72)	452/702 (64)	877/1364 (64)
Unknown	11/279 (4)	3/383 (1)	36/702 (5)	49/1364 (4)
GBV-C viral load <sup>a</sup>				
Log <sub>10</sub> copies/mL	5.5 (4.1–6.5)	8.0 (7.5–8.4)	7.9 (6.8–8.3)	7.4 (5.8–8.1)
Participants evaluated, no.	88	58	116	262
GBV-C genotype <sup>a,b</sup>				
2A	44/88 (50)	24/58 (41)	31/110 (28)	99/256 (39)
2B	0/88 (0)	2/58 (3)	3/110 (3)	5/256 (2)
3	26/88 (30)	19/58 (33)	48/110 (44)	93/256 (36)
4	8/88 (9)	11/58 (19)	22/110 (20)	41/256 (16)
Multiple	10/88 (11)	2/58 (3)	6/110 (5)	18/256 (7)
Age, years				
Value	22 (20–26)	24 (22–28)	26 (23–30)	25 (22–28)
Participants evaluated, no.	279	383	702	1364
Gravidity				
Value	1 (1–2)	2 (1–2)	2 (1–2)	2 (1–2)
Participants evaluated, no.	279	383	701	1363
Antiretroviral regimen				
None	279/279 (100)	195/383 (51)	...	474/1364 (35)
Maternal ZDV	...	188/383 (49)	...	188/1364 (14)
Maternal and infant ZDV	...	...	373/702 (53)	373/1364 (27)
Infant ZDV	...	...	119/702 (17)	119/1364 (9)
Maternal and infant ZDV + nevirapine	...	...	210/702 (30)	210/1364 (15)
HIV viral load at delivery				
Log <sub>10</sub> copies/mL	4.3 (3.9–4.9)	4.2 (3.7–4.8)	3.8 (3.2–4.4)	4.1 (3.5–4.6)
Participants evaluated, no.	278	383	700	1361
CD4 <sup>+</sup> count at delivery				
Cells/mm <sup>3</sup>	450 (330–570)	380 (267–530)	403 (271–552)	410 (280–556)
Participants evaluated, no.	275	383	700	1358
Maternal NK cells, % at delivery				
Value	11 (8–14)	11 (7–15)	10 (7–13)	10 (7–14)
Participants evaluated, no.	275	383	700	1358
HIV subtype <sup>b</sup>				
E	260/273 (95)	310/350 (89)	527/596 (88)	1097/1219 (90)
B	10/273 (4)	22/350 (6)	45/596 (8)	77/1219 (6)
E/B	3/273 (1)	16/350 (5)	11/596 (2)	30/1219 (2)
BR/MN/C	...	2/350 (1)	13/596 (2)	15/1219 (1)
<b>Delivery characteristic</b>				
Cesarean delivery	33/279 (12)	56/383 (15)	179/702 (26)	268/1364 (20)
Labor				
Duration, h	7.0 (4.0–10.0)	8.5 (5.0–14.7)	7.3 (4.4–11.3)	7.6 (4.6–11.9)
Participants evaluated, no.	250	383	605	1238
Membrane rupture				
Duration, h	2.0 (1.0–4.0)	1.9 (0.3–4.6)	0.5 (0.1–2.8)	1.0 (0.2–3.9)
Participants evaluated, no.	266	380	695	1341
<b>Infant characteristic</b>				
Perinatal HIV infection <sup>c</sup>	68/279 (24)	55/383 (14)	53/702 (8)	176/1364 (13)
Perinatal GBV-C infection <sup>a,c</sup>	21/81 (26)	29/54 (54)	51/110 (46)	101/245 (41)
Birth at <37 weeks	12/277 (4)	9/383 (2)	58/702 (8)	79/1362 (6)
Birth weight <2500 g	28/279 (10)	29/383 (8)	75/702 (11)	132/1364 (10)

**NOTE.** Data are proportion (%) of subjects or median (interquartile range), unless otherwise indicated. Peri-1, Peri-2, and Peri-3 were 3 perinatal HIV transmission studies that did not involve breast-feeding, conducted jointly by the Thailand Ministry of Public Health and the US Centers for Disease Control and Prevention at 2 large Bangkok hospitals. GBV-C, GB virus C; ZDV, zidovudine.

<sup>a</sup> GBV-C RNA<sup>+</sup> mothers only.

<sup>b</sup> Subjects with nontypable virus excluded.

<sup>c</sup> For those with known results.



**Figure 1.** Determining maternal and infant GB virus C (GBV-C) status. There were 1498 women enrolled in the 3 studies by 31 March 2004. A total of 1440 women had maternal plasma specimens available; 1364 of these women had infants whose HIV status was known. +, positive; -, negative.

causal pathway between receipt of ART and MTCT of HIV, it was retained in the model and improved model fit; removing it increased the estimated effect of ART as expected, but did not change any other estimates. Other variables that significantly increased the risk of MTCT of HIV in multivariate modeling included high maternal HIV viral load at delivery, no maternal receipt of ART, percentage of maternal NK cells at delivery <5%, vaginal delivery, duration of membrane rupture greater than 4 h, and female sex of the infant. We observed no significant difference in median CD4<sup>+</sup> counts between infants infected with GBV-C but not infected with HIV and infants not infected with either GBV-C or HIV (at birth, 2170 and 2179, respectively [ $P = .80$ ]; at 2 months, 2335 and 2346 [ $P = .73$ ]; at 6 months, 2670 and 2360 [ $P = .07$ ]).

## DISCUSSION

In 3 separate studies of HIV-infected pregnant women in Thailand, we found that infants who acquired GBV-C from their mothers had lower rates of MTCT of HIV, compared with infants who were not infected with GBV-C. This association was statistically significant in both the univariate and multivariate analysis of the combined studies. Neither the presence of maternal GBV-C RNA nor the presence of GBV-C antibody affected the risk of MTCT of HIV.

Ours is the first study to observe an association between infant GBV-C infection and MTCT of HIV. One possible explanation for the observed association between infant GBV-C infection and lower rates of HIV acquisition could have been confounding. High maternal GBV-C viral load is associated with both increased transmission of GBV-C to the infant (in our study [data not shown] and others [18, 19]) and a lower level of plasma HIV RNA (in our study and others [20–22]), which is the main determinant of MTCT of HIV. Thus, infants who are infected with GBV-C could appear less likely to be HIV infected. However, because we adjusted for maternal HIV viral load in our multivariate analysis, the observed association is unlikely to be due to confounding by maternal HIV viral load.

Although we observed a statistical association between infant GBV-C infection and MTCT of HIV, we cannot conclude that the relationship is causal. We do not know the direction or mechanism for the observed effect. It is possible that infant HIV infection prevents infant GBV-C acquisition rather than the reverse, or that a third factor is responsible for the low observed rates of both HIV and GBV-C transmission. In adults, it is unlikely that either GBV-C or HIV prevents acquisition of the other virus, given the high coinfection rates observed [2–4] and the lack of an association between GBV-C viremia and HIV acquisition [23] as well as the similar rates of GBV-C acquisition and

**Table 2. Association of infant GB virus C (GBV-C) infection status with mother-to-child transmission of HIV in Bangkok, Thailand, according to the perinatal study in which subjects originally participated.**

Study, child GBV-C status	HIV-infected infants, proportion (%)	OR (95% CI)	P
<b>Peri-1, no ART</b>			
RNA <sup>+</sup>	0/21 (0)	0 (0–0.60)	.005 <sup>a</sup>
Negative	62/248 (25)	1.0 <sup>b</sup>	
<b>Peri-2, placebo</b>			
RNA <sup>+</sup>	0/7 (0)	0 (0–2.95)	.35 <sup>a</sup>
Negative	37/187 (20)	1.0 <sup>b</sup>	
<b>Peri-2, ZDV</b>			
RNA <sup>+</sup>	1/22 (5)	0.43 (0.05–3.45)	.70 <sup>a</sup>
Negative	16/162 (10)	1.0 <sup>b</sup>	
<b>Peri-3</b>			
RNA <sup>+</sup>	1/51 (2)	0.25 (0.03–1.85)	.25 <sup>a</sup>
Negative	47/635 (7)	1.0 <sup>b</sup>	
<b>Total</b>			
RNA <sup>+</sup>	2/101 (2)	0.13 (0.03–0.54) <sup>c</sup>	<.001
Negative	162/1232 (13)	...	...

**NOTE.** Peri-1, Peri-2, and Peri-3 were 3 perinatal HIV transmission studies that did not involve breast-feeding, conducted jointly by the Thailand Ministry of Public Health and the US Centers for Disease Control and Prevention at 2 large Bangkok hospitals between 1992 and 2004. ART, antiretroviral therapy; CI, confidence interval; OR, odds ratio; RNA<sup>+</sup>, RNA positive; ZDV, zidovudine.

<sup>a</sup> Fisher exact test.

<sup>b</sup> Reference.

<sup>c</sup> OR adjusted for study.

clearance in HIV-infected and -uninfected women [24] observed in 2 recent studies. The same may not be true in the context of MTCT. Two additional studies have also noted a low prevalence of GBV-C infection among HIV-infected infants [25, 26]. While HIV-infected children appear to acquire GBV-C, as the prevalence of GBV-C infection was observed in one study to be higher in older HIV-infected children, compared with younger HIV-infected children [26], it is not known whether HIV-infected children acquire GBV-C at the same rate as HIV-uninfected children. No studies have examined whether GBV-C infection prevents HIV acquisition in infancy or childhood.

The precise temporal relationship between infant acquisition of HIV and infant GBV-C infection is unknown, but it appears that both HIV and GBV-C are acquired at similar times. In populations that do not receive ART and who do not breast-feed, it is thought that most transmission of HIV occurs late in pregnancy or during delivery [27]. HIV-1 can be detected by DNA PCR at birth in 25%–38% of HIV-infected infants and by 2 months in the remainder [28–30]. In our study, GBV-C RNA was detectable by PCR at birth in 3 (21%) of 14 infants in Peri-1 who tested positive for GBV-C RNA (data not shown). In several other small studies of vertical transmission of GBV-C, the virus was detectable by PCR in 10%–100% of infants at birth [14, 31, 32] and in all infants by 2–3 months of age [13, 14, 33], most

likely indicating a similar timing of vertical transmission for both HIV and GBV-C. Cesarean delivery was associated with reduced rates of GBV-C vertical transmission in our study and 2 others [18, 19], which is also suggestive of transmission at delivery.

GBV-C–infected infants are most likely infected through vertical transmission. Most, but not all, studies have observed high GBV-C sequence homology in mother-infant pairs [14, 19, 34, 35] and no GBV-C RNA in plasma samples from infants born to RNA-negative mothers [13, 31, 34, 36]. In our study, none of the infants born to 38 GBV-C RNA–indeterminate mothers tested positive for GBV-C RNA (figure 1). Although it is theoretically possible that infants in our study acquired GBV-C from their mother or someone else after birth (GBV-C RNA has been isolated from saliva [37] but not from breast milk [38]), household transmission in infancy is thought to occur rarely, if at all [34, 39], and in any case is unlikely in our study, given the short interval from birth to sample collection.

**Table 3. Association of maternal GB virus C (GBV-C) infection status with mother-to-child transmission of HIV in Bangkok, Thailand, according to the perinatal study in which subjects originally participated.**

Study, maternal GBV-C status	HIV-infected infants, proportion (%)	OR (95% CI)	P
<b>Peri-1</b>			
RNA <sup>+</sup>	21/88 (24)	0.83 (0.45–1.52)	.54
Antibody <sup>+</sup>	3/31 (10)	0.28 (0.08–0.98)	.036
Negative	41/149 (28)	1.0 <sup>a</sup>	
<b>Peri-2, placebo</b>			
RNA <sup>+</sup>	7/24 (29)	2.00 (0.75–5.37)	.17
Antibody <sup>+</sup>	6/29 (21)	1.27 (0.47–3.46)	.64
Negative	24/141 (17)	1.0 <sup>a</sup>	
<b>Peri-2, ZDV</b>			
RNA <sup>+</sup>	3/34 (9)	0.99 (0.26–3.73)	>.99 <sup>b</sup>
Antibody <sup>+</sup>	2/17 (12)	1.37 (0.28–6.70)	.66 <sup>b</sup>
Negative	12/135 (9)	1.0 <sup>a</sup>	
<b>Peri-3</b>			
RNA <sup>+</sup>	7/116 (6)	0.79 (0.34–1.83)	.58
Antibody <sup>+</sup>	9/99 (9)	1.23 (0.57–2.65)	.60
Negative	34/452 (8)	1.0 <sup>a</sup>	
<b>Total</b>			
RNA <sup>+</sup>	38/262 (15)	0.94 (0.62–1.42) <sup>c</sup>	.77
Antibody <sup>+</sup>	20/176 (11)	0.90 (0.54–1.50) <sup>c</sup>	.69
Negative	111/877 (13)	1.0 <sup>a</sup>	

**NOTE.** Peri-1, Peri-2, and Peri-3 were 3 perinatal HIV transmission studies that did not involve breast-feeding, conducted jointly by the Thailand Ministry of Public Health and the US Centers for Disease Control and Prevention at 2 large Bangkok hospitals between 1992 and 2004. Antibody<sup>+</sup>, antibody positive; CI, confidence interval; OR, odds ratio; RNA<sup>+</sup>, RNA positive; ZDV, zidovudine.

<sup>a</sup> Reference.

<sup>b</sup> Fisher exact test.

<sup>c</sup> OR adjusted for study.

**Table 4. Variables associated with mother-to-child transmission of HIV in 1364 mother-infant pairs, Bangkok, Thailand.**

Variable	HIV-infected infants, proportion (%)	aOR adjusted for study only (95% CI)	<i>P</i>	aOR final multivariate model (95% CI)	<i>P</i>
<b>Child GBV-C status</b>					
RNA positive	2/101 (2)	0.13 (0.03–0.53)	<.001	0.14 (0.03–0.58)	.007
Negative	162/1232 (13)	1.0 <sup>a</sup>			
<b>Maternal HIV viral load at delivery, log<sub>10</sub> viral copies/mL</b>					
≥5.0	61/170 (36)	12.03 (7.00–20.67)	<.001	3.33 (2.52–4.41) per log <sub>10</sub> increase	<.001
4.0–4.9	92/552 (17)	4.44 (2.74–7.19)	<.001		
<4.0	23/639 (4)	1.0 <sup>a</sup>			
<b>Maternal receipt of ART</b>					
Yes	53/771 (7)	0.41 (0.26–0.63)	<.001	0.50 (0.34–0.75)	<.001
No	123/593 (21)	1.0 <sup>a</sup>			
<b>Maternal CD4<sup>+</sup> count at delivery, cells/mm<sup>3</sup></b>					
≥500	47/460 (10)	0.35 (0.20–0.63)	<.001		
200–499	101/745 (14)	0.56 (0.35–0.92)	.02		
<200	27/153 (18)	1.0 <sup>a</sup>			
<b>Maternal NK cell percentage at delivery</b>					
<5	18/91 (20)	1.95 (1.12–3.39)	.02	2.50 (1.35–4.63)	.004
≥5	157/1267 (12)	1.0			
<b>Cesarean delivery</b>					
Yes	20/268 (7)	0.59 (0.36–0.97)	.03	0.54 (0.31–0.94)	.03
No	156/1096 (14)	1.0 <sup>a</sup>			
<b>Duration of ruptured membranes</b>					
>4 h	54/307 (18)	1.58 (1.10–2.25)	.01	1.75 (1.17–2.61)	.007
≤4 h	118/1034 (11)	1.0 <sup>a</sup>			
<b>Prematurity</b>					
<37 weeks	15/79 (19)	2.16 (1.17–4.01)	.01		
≥37 weeks	159/1283 (12)	1.0 <sup>a</sup>			
<b>Infant sex</b>					
Female	102/693 (15)	1.40 (1.01–1.94)	.04	1.50 (1.04–2.17)	.03
Male	174/671 (11)	1.0 <sup>a</sup>			
<b>Low birth weight</b>					
<2500 g	24/132 (18)	1.67 (1.02–2.72)	.04		
≥2500 g	152/1232 (12)	1.0 <sup>a</sup>			
<b>Maternal CD8<sup>+</sup> percentage at delivery</b>					
<55%	113/1020 (11)	0.48 (0.34–0.68)	<.001		
≥55%	61/337 (18)	1.0 <sup>a</sup>			

**NOTE.** The number of infants evaluated in each category does not necessarily add up to 1364 because data were missing for some participants. aOR, adjusted odds ratio; ART, antiretroviral therapy; CI, confidence interval; GBV-C, GB virus C.

<sup>a</sup> Reference.

Several plausible biologic mechanisms have been suggested for GBV-C interference with HIV replication. In cell cultures simultaneously coinfecting with GBV-C and HIV, the replication of HIV was reduced by 49%, compared with cell cultures infected with HIV alone, whereas cell cultures that were coinfecting with GBV-C prior to being infected with HIV showed a 99% reduction in viral replication 6 days after infection [6]. GBV-C infection in cell culture also reduced surface expression of the

CCR5 receptor and induced production of chemokine ligands for the CCR5 receptor, including RANTES, MIP-1 $\alpha$ , MIP-1 $\beta$ , and SDF-1, which appeared to decrease HIV replication, although such effects may not be reproducible in vivo [7].

There may also be mechanisms by which HIV interferes with GBV-C susceptibility or persistence and it has been hypothesized that the declining CD4<sup>+</sup> count in HIV progression results in loss of GBV-C [2]. Rapidly progressing HIV is unlikely to be

responsible for the lack of coinfection observed in our studies, as there was no GBV-C coinfection among HIV-infected children in Peri-1, regardless of the rate of HIV progression (approximately 25% were rapid progressors, whereas 75% were slow or intermediate progressors [40]). A similar bias is also unlikely to have been introduced by the 13 missing infant specimens from infants born to GBV-C RNA–positive mothers, because even if 41% of the 6 HIV-infected and 7 HIV-uninfected infants had acquired GBV-C, our result would still be significant (OR, 0.25 [95% CI, 0.09–0.70]). In addition, such an effect would be unlikely to occur through CD4<sup>+</sup> cell depletion, because although median CD4<sup>+</sup> counts were lower in Peri-1 rapid progressors, compared with slow progressors, at 2 months of age (1510 and 2610 cells/mm<sup>3</sup>, respectively [40]), they were presumably sufficient to support GBV-C replication. Also, among HIV-uninfected infants in our study, we observed no difference in median CD4<sup>+</sup> counts between GBV-C–infected and GBV-C–uninfected infants. Thus, in our study, there was no evidence that rapid HIV progression or the absolute number of CD4<sup>+</sup> cells determined GBV-C susceptibility or persistence in early infancy.

It is possible that maternal GBV-C RNA has a weaker effect on MTCT of HIV than was detectable by our study. With a population of 262 women positive for GBV-C RNA among 1364 HIV-infected women (19%) and a 13% overall rate of MTCT of HIV, we had the power to detect a 65% reduction in MTCT of HIV (OR, 0.52) with presence of maternal GBV-C RNA. Since initiating this study, 2 other studies [41, 42] also failed to demonstrate an effect of maternal GBV-C infection on MTCT of HIV, although the latter study did observe a borderline beneficial effect in women after highly active ART became available. Two additional studies [36, 43] were too small to draw definitive conclusions. In our study, GBV-C RNA also appeared somewhat more protective against MTCT of HIV in women who received ART, compared with women who did not, although the difference was not significant.

ART appears to play an important role in GBV-C infection. In our study, HIV and GBV-C viral loads were inversely correlated only among women who received ART. A recent longitudinal study also observed that HIV replication decreased and GBV-C replication increased among coinfecting individuals who received highly active ART [44].

The higher prevalence of GBV-C viremia observed in Peri-1 is noteworthy, especially given that lower viral loads would presumably have made detecting GBV-C more difficult. The reasons for the lower viral load observed in Peri-1 are unknown, but they may include length of storage of specimens, the absence of ART, or other changes in the population or epidemiology of GBV-C during the study period.

Our study had several limitations. Because all study subjects were Thai, the generalizability of our findings may be limited to certain populations, HIV subtypes, or GBV-C genotypes. The

262 women infected with GBV-C in our study had GBV-C genotypes 2, 3, or 4, which may differ in their interaction with HIV, compared with genotypes 1 and 5, which are found predominantly in Africa [45]. A limitation of GBV-C studies in general is the lack of a gold standard test for the detection of GBV-C RNA. As random measurement error tends to bias the odds ratio toward the null, measurement error might have masked a true association between maternal GBV-C infection and MTCT of HIV. In the unlikely event that measurement error was associated with the outcome (MTCT of HIV), it is possible our results could be biased in either direction. This is unlikely, however, given the strong association we observed with infant GBV-C infection. We did not test infants of GBV-C RNA–negative mothers, and, because the sensitivity of the GBV-C test is less than perfect, some women and their infants may have been incorrectly classified as GBV-C RNA negative. If the sensitivity of the GBV-C test was 80%, then it is possible that 66 women and 27 of their untested infants (assuming 41% MTCT of GBV-C) were misclassified as negative. However, even if 100 infants were misclassified as negative and 13 (13%) of these infants were HIV infected (the rate observed in our study), the observed association would still be significant (OR, 0.53 [95% CI, 0.30–0.93]). In addition, the observed association was also present when our analysis was restricted to infants born to GBV-C RNA–positive mothers, all of whom had samples tested for GBV-C RNA (OR, 0.08 [95% CI, 0.02–0.34]).

In conclusion, we observed no association between maternal GBV-C infection and MTCT of HIV, but we found a strong inverse association between infant GBV-C infection and MTCT of HIV. In the context of MTCT, the interaction between GBV-C and HIV appears to occur at or around the time of delivery and is independent of infant CD4<sup>+</sup> count. Our results are robust and do not change with adjustment for potential sources of bias including confounding, measurement error, and missing specimens. Nevertheless, our findings need to be confirmed by other studies, and the underlying causal pathway and mechanisms need to be identified. GBV-C may, for example, influence the cytokine milieu in such a way as to reduce the risk of infant HIV acquisition. If infant GBV-C infection is ultimately found to prevent HIV acquisition, avenues should be explored for a potential therapeutic role of GBV-C or its associated mechanisms in the prevention of MTCT of HIV.

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