

CONCISE COMMUNICATION

Human Immunodeficiency Virus Type 1 Infection in Twin Pairs Infected at Birth

Robert J. Biggar,¹ Michelle Janes,³ Richard Pilon,³
 Reena Roy,⁴ Robin Broadhead,⁵ Newton Kumwenda,⁶
 Taha E. T. Taha,² and Sharon Cassol⁷

¹Viral Epidemiology Branch, National Cancer Institute, Bethesda, and ²Johns Hopkins School of Public Health, Baltimore, Maryland; ³University of Ottawa, Ottawa, Canada; ⁴St. Louis County Police Crime Laboratory, St. Louis, Missouri; ⁵Department of Paediatrics, University of Malawi, and ⁶Johns Hopkins University/University of Malawi Project, Blantyre; ⁷The Africa Centre and Nelson R. Mandela School of Medicine, University of Natal, Durban, South Africa

Host genetic factors may influence the course of human immunodeficiency virus (HIV) infection. In Blantyre, Malawi, polymerase chain reaction was used to identify twin pairs who were concordantly HIV-1-infected in utero or perinatally and then to examine strain divergence or virus levels in identical and fraternal twin pairs. Among 315 twin pairs, both infants in 14 fraternal and 5 identical pairs were found to be infected at the same visit. Among 10 pairs, HIV-1 sequences were determined for both infants at ≥ 1 time point. HIV levels had a common profile in both fraternal and identical twin pairs. Identical twins were not always infected by the same quasi species, indicating that their mothers had multiple quasi species capable of infecting their infants. Subsequent viral divergence appears to depend on quasi-species stability rather than on genetically controlled host immune responses. Thus, given infection, factors intrinsic to HIV-1 are more important than host genetics in viral evolution.

Genetic factors such as chemokine receptor genes play a major role in susceptibility to human immunodeficiency virus (HIV) type 1 infection [1], but the role of genetics is hard to determine. In vitro studies indicate that genetics could be important [2, 3]; however, in the more complicated in vivo situation, the outcome might be influenced by donor genetics, quasi-species variation, exposure routes, inoculum of the virus, age at infection, coinfections, passively acquired antibodies in newborns, and the timing of postinfection evaluations.

To address these issues in one setting, we examined twin pairs perinatally coinfecting with HIV-1 in Blantyre, Malawi. The primary objective of the study was to evaluate the importance of birth order in these twins. However, the size of the study permitted an in-depth evaluation of virus level changes and quasi-species sequence variation in vivo in the unusual circumstance of coinfecting twin pairs. We further evaluated their genetic status to determine whether the twins were identical or fraternal, although even nonidentical twins, being siblings, share 50% genetic similarity on average.

Methods

Enrollment procedures were similar to those described in our other studies in Malawi [4, 5]. Between 1994 and 1999, multiple birth mothers delivering at Queen Elizabeth Central Hospital, Blantyre, were enrolled. HIV status of the mother was determined by antibodies in umbilical cord blood by use of reactivity in 2 commercial HIV test kits. Infants were sampled at birth (using umbilical cord blood) and by heel stick when they returned (per schedule at ages 6, 12, 26, and 52 weeks). Whole-blood samples all were obtained as dried blood spots. Samples were screened for HIV DNA by established chelex methods that work well for detecting HIV [4, 5]. For determining virus levels, HIV amplification and detection were done by the Organon Teknika Nuclisens HIV-1 RNA QT (quantitative) and QL (qualitative) assays, adapted for use with dried blood spot specimens. For genotypic analyses, nucleic acids were extracted from dried blood spots by use of the NucliSens isolation kit (Organon Teknika). We used a nested polymerase chain reaction (PCR) to amplify a 771-bp outer and a 606-bp inner fragment, spanning the C2 to C5 regions of the HIV-1 envelope gene. Alternative primers giving rise to shorter fragments (341 bp) were used if no result was obtained from the longer fragment PCRs. Products were sequenced on an Applied Biosystems 377 sequencer (PE Biosystems).

Sequences were aligned by use of Clustal X software. Differences are described as the percentage of pairwise nucleotide diversity between samples. Phylogenetic analyses and tree construction used the neighbor-joining methods of Saitou and Nei [6], based on gap-stripped sequences, to avoid disproportionate weighing of these loci. For comparative purposes, 1 clade B sequence and 3 clade C sequences selected from the HIV-1 subtypes in the HIV Sequence

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Reprints or correspondence: Dr. Robert J. Biggar, National Cancer Institute, Viral Epidemiology Branch, 6120 Executive Blvd., Bethesda, MD 20852 (biggarb@epndce.nci.nih.gov).

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Database are presented. Bootstrap values were based on 1000 replications and exclude reference sequences. When sequences from subjects were identical, we entered only a single sequence to represent that group, to avoid artifacts from random allocation of positions in identical samples. Sequences derived from these subjects have been entered in GenBank (National Institutes of Health) and are listed in an Appendix published only in the electronic edition of the *Journal* (<http://www.journals.uchicago.edu/JID/>). Samples from same-sex twin pairs were tested with the AmpF/STR Profiler Plus (Cofiler) and Identifiler PCR amplification kits (Applied Biosystems) and with the GenePrint PowerPlex 16 System (Promega). Both kits identified the same twin pairs as identical. $P < .05$ was considered to be statistically significant.

Results

During the study period, 351 of 1124 women giving birth to twins were HIV infected. Of 315 live-born pairs with samples at the same visit, 22 pairs were concordantly infected with HIV-1. In 3 pairs, infection occurred at different ages. The remaining 19 twin pairs were found to be concordantly infected at the same visit and form the basis of this study. Nine pairs were infected in utero (6 fraternal and 3 identical pairs), and 10 others were infected perinatally (7 fraternal and 2 identical pairs). One pair, found to be infected at 8 weeks, lacked cord blood and was assumed to be perinatally infected, because perinatal infection is twice as common as in utero infection.

HIV infection risk and virus levels. Concordant infection status was more frequent than expected both for in utero (κ , 0.43; 95% confidence interval [CI], 0.22–0.63) and perinatal (κ , 0.33; 95% CI, 0.01–0.52) infections, respectively. Even so, we cannot be certain that the twin mates were necessarily infected at the same time or by the same quasi species. In cord blood samples, the difference between \log_{10} virus levels in identical or fraternal twin pairs was not significant ($P = .19$). Twin mates often had similar cord blood HIV level (figure 1). Similarly, all subjects who were followed longitudinally showed similar patterns in both identical and fraternal twins, regardless of whether the infection was acquired in utero (not shown because of uncertainty about the exact time of infection) or perinatally (figure 2).

HIV sequence variation. We attempted sequence analysis on all PCR-positive samples. The mean (\pm SD) virus level in samples was $\log_{10} 4.4 \pm 0.7$ HIV copies/mL when we could not sequence amplicons, compared with $\log_{10} 5.4 \pm 0.6$ HIV copies/mL when we obtained sequences. Among those sequenced, there was no apparent relationship between virus level and the amount of sequence variation, either in the same infant followed over time or in samples from the twin mates at the same visit.

All sequences were from clade C HIV. The V3 sequences had limited variability that was not associated with basic amino acid substitutions (except at position 13), characteristics typical of HIV in Malawi and shown elsewhere [7] to be associated with macrophage-tropic, nonsyncytium-inducing strains. We

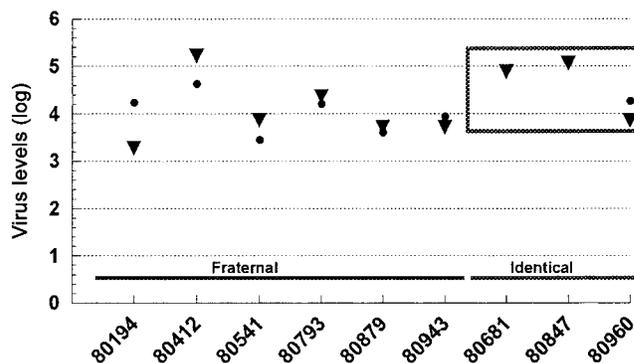


Figure 1. Human immunodeficiency virus type 1 levels in twin pairs concordantly infected in utero, by zygosity status. Nos. refer to the twin pair identifiers.

measured sequence variation as the percentage differences between the sequenced virus in each sample from the calculated central divergence point for both sequences (figure 3). The reference clade B sequence (Bweau) diverged 14.2% from the closest node to any other sequence and 15.4% from the most central node for this group (circled junction). For comparison, the 3 reference clade C sequences diverged ~6.2%–9.5% from the central node. Most individual infants in this study were also ~8%–9% divergent from the central node (range, 4.8%–11.9%).

Figure 3 shows sequence divergence between members of twin pairs over time. Among twin pairs with sequenced samples from both infants at the same visit, both infants clearly were infected with closely related viruses in many cases. The bootstrap value was 97.6%–100% for 8 of 10 twin pairs. However, for twin pairs 80681 and 80816, the bootstrap values were relatively low (39.6% and 51.1%, respectively). Various patterns can be seen in subsequent samples from the same individuals. In some pairs, both twins had stable quasi species over long periods. In others, the quasi species diverged in 1 or both infants. These patterns did not appear to be affected by identical or fraternal twin pair status. Viruses sequenced in fraternal twin pairs infected in utero showed considerable difference in their first sequenced samples (80793 at birth; 20987 at 8 weeks) as did viruses from pair 80816 at 7 weeks after perinatal infection. However, identical twin pair 80681 also showed considerable divergence, although the first samples sequenced were at 28 weeks after in utero infection.

Divergence between viruses in the 5 identical twin pairs tested at the same visit averaged 1.2% (range, 0–4.5%; mean age at visit, 16 weeks). However, 3 of the 5 comparisons in identical twins were made between paired samples obtained at different times in the same twin set (80960), a set that appeared to be infected with an exceptionally stable virus. Divergence in 7 fraternal pairs averaged 2.7% (range, 0–4.6%; average age at visit, 12 weeks), excluding 1 pair (26541) with sequences obtained at 130 weeks, whose sequences diverged 2.5% from their common node.

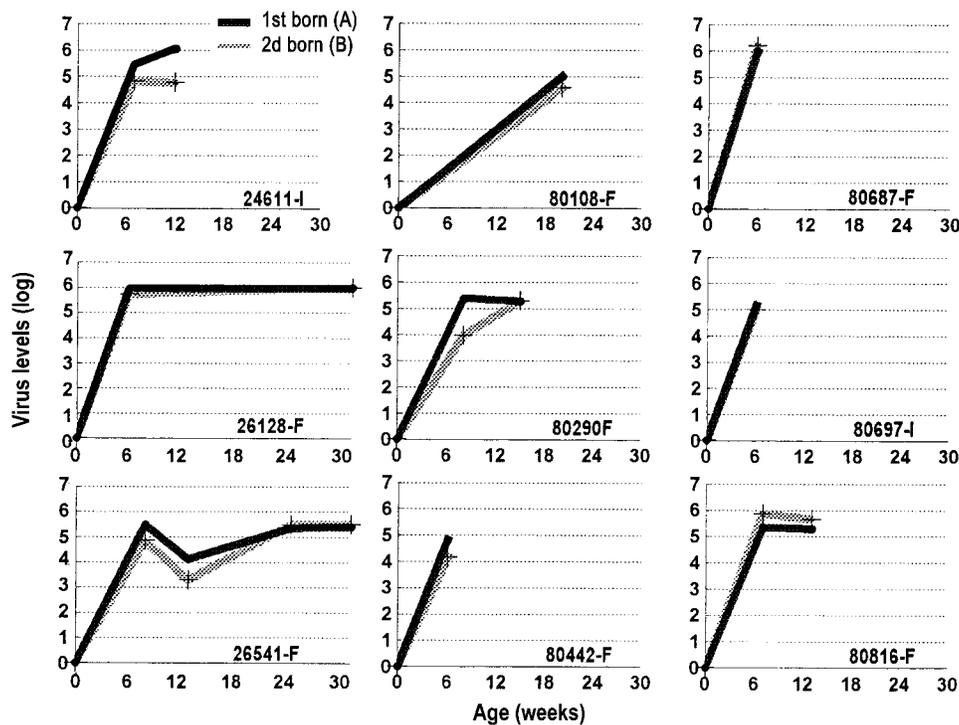


Figure 2. Human immunodeficiency virus type 1 levels in perinatally infected twin pairs followed longitudinally. The zygosity status is shown in the suffix I (identical) or F (fraternal) attached to the identity numbers.

Among infants followed over time, sequence divergence averaged 0.08% per week among 5 infants in 4 pairs (range, 0.01%–0.16% per week) from first to last visits. In an additional twin pair (80816), between weeks 6 and 13, the strain changed 0.01% per week in 1 infant but 1.3% in the twin mate. We postulate that the latter infant might have been infected by at least 2 different quasi species, 1 amplified at week 6 and the other at week 13. In pair 26541, 1 infant had sequences determined at both 25 and 130 weeks, during which time the sequence changed 2.3% (0.025% per week). Sequence changes did not appear to be faster in those with higher virus levels and did not appear to accelerate with time from infection.

Discussion

The high frequency of concordance in the HIV infection status of these twin pairs suggests that infection risk was determined largely by the mother’s virus levels, with mothers who had high HIV-1 levels being likely to infect both members of their twin pair [9, 10]. Elsewhere, we and others show that the risk of in utero infection is half of perinatal infection [11, 12]. In the present study, the apparently high proportion of infants infected in utero versus those infected perinatally occurred because more subjects had paired cord blood samples than later paired samples.

The pattern of viremia appeared to be similar for all infants,

whether measured within members of fraternal or identical twin pairs. Among in utero–infected infants, the high virus levels imply that infection occurred well before delivery. Among perinatally infected infants, probably infected at delivery, HIV-1 levels peak within the first month but stabilize by age 6 weeks [4, 5]. Although these levels are typical of those seen in singleton infants at the same age [4, 5], the minor perturbations of the levels with twin pairs were also remarkably similar, possibly because of similarity to variation in sample collection and storage conditions, which were the same for both infants but might have varied between visit dates. We had predicted that, if host genetics influenced control of viral replication, then fraternal twins, being genetically different, would have more variation in virus levels than identical twins. However, we did not confirm this hypothesis, finding that levels were similar in both identical and fraternal twin pairs.

As expected for this region of Africa, all sequences were from clade C HIV-1 [8]. The patterns of HIV-1 genomic changes in the viruses of twin pairs are diverse. In the majority, the first viruses varied little in the region we sequenced, suggesting infection by the same or closely related quasi species. However, 2 pairs had a large divergence in their first sequenced samples. This difference suggests that different quasi species could have caused infection in some infants, although we cannot exclude a rapid divergence from a single unstable variant. Over time, different patterns of viral divergence occurred in both identical and fraternal twin pairs. Some pairs had stable HIV-1 quasi

4. Biggar RJ, Broadhead R, Cormier M, et al. Virus levels in newborn African infants undergoing primary HIV-1 infections. *AIDS* **2001**;15:1311-3.
5. Biggar RJ, Janes M, Pilon R, et al. Virus levels in untreated African infants infected with human immunodeficiency virus type 1. *J Infect Dis* **1999**;180:1838-43.
6. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **1987**;4:406-25.
7. Ping LH, Nelson JAE, Hoffman IF, et al. Characterization of V3 sequence heterogeneity in subtype C human immunodeficiency virus type 1 isolates from Malawi: underrepresentation of X4 variants. *J Virol* **1999**;73:6271-81.
8. Carr J, Foley B, Leitner T, Salminen M, Korber B, McCutchan F. Human retroviruses and AIDS: reference sequences representing the principal genetic diversity of HIV-1 in the pandemic: a compilation and analysis of nucleic acid and amino acid sequences. Los Alamos, NM: Los Alamos National Laboratory, **1998**.
9. Fang G, Burger H, Grimson R, et al. Maternal plasma human immunodeficiency virus type 1 RNA level: a determinant and projected threshold for mother-to-child transmission. *Proc Natl Acad Sci USA* **1995**;92:12100-4.
10. Thea DM, Steketee RW, Pliner V, et al. The effect of maternal viral load on the risk of perinatal transmission of HIV-1. New York City Perinatal Transmission Collaborative Study Group. *AIDS* **1997**;11:437-44.
11. Bertolli J, St.Louis ME, Simonds RJ, et al. Estimating the timing of mother-to-child transmission of human immunodeficiency virus in a breast-feeding population in Kinshasa, Zaire. *J Infect Dis* **1996**;174:722-6.
12. Simonon A, Lepage P, Karita E, et al. An assessment of the timing of mother-to-child transmission of human immunodeficiency virus type 1 by means of polymerase chain reaction. *J Acquir Immune Defic Syndr* **1994**;7:952-7.
13. Wolinsky SM, Wike CM, Korber BT, et al. Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. *Science* **1992**;255:1134-7.