Lack of Evidence for Prolonged Double–Long Terminal Repeat Episomal HIV DNA Stability In Vivo

To the Editor:

Low-level HIV replication seems to persist during long-term effective highly active antiretroviral therapy (HAART). The sites of this replication are unclear, and the resulting viremia would be too low to detect with conventional procedures. It has been proposed that latently infected resting CD4 T cells release the virus on activation or that viral replication persists in anatomic sanctuaries poorly accessible to antiretroviral drugs. Double-long terminal repeat (LTR) episomal HIV DNA arises when reverse-transcribed viral DNA fails to integrate the host genome and is circularized. It has been suggested that these circular DNA forms might serve as a marker of recent cell infection on the basis of their observed lability in vitro and in vivo.[1–3] Other in vitro studies have suggested that these DNA circles are highly stable and may persist indefinitely should the infected cells survive and remain in the compartments being sampled, however.[4,5] This implies that HIV double-LTR DNA circles found in patients on long-term effective HAART could originate mostly from the pre-HAART period of high-level replication. In an attempt to settle this issue, we compared sequences of the hypervariable gp120 V3 loop in plasma HIV RNA at the outset of first-line HAART with those of peripheral blood mononuclear cell (PBMC) episomal HIV DNA during long-term effective HAART (Fig. 1).

We selected 3 patients who were highly adherent to HAART and who had plasma HIV-1 RNA <20 copies/mL (Amplicor Ultrasensible; Roche Diagnostics, Meylan, France) for 2 to 5 years without a single detected viral blip. Episomal DNA was isolated from PBMC genomic DNA (QIAprep Spin Mini prep Kit; Qiagen, Hilden, Germany), and polymerase chain reaction (PCR) assay was performed with a primer pair targeting the 2-LTR circle junctions (5′-CAGATCTGGTCTAACCAGAGA-3′ and 5′-GTAACTAGAGATCCCTCAGAC-3′).[1,2] The corresponding PCR products were cloned and sequenced. The sequences of the 2-LTR circle junction were determined in each patient and then used to design 1 or 2 specific primers, which were pooled for use. Episomal DNA was then PCR amplified with the 2-LTR junction primer(s) and the Env13 primer (5′-CCACTCTATTTTGTGCATCAGA-3′) located in the *Env* gene. The sequence amplified included the C2 to V4 regions of gp120. Nested PCR was applied to the resulting purified PCR product by using a pair of primers located in the V3 loop of gp120 (B1: 5′-ACACATGGAATTAGGCCAGT-3′ and B2: 5′-CTGCCACATGTTTATAATTTG-3′).

RNA was extracted from frozen plasma obtained 3 to 4 weeks before the outset of first-line HAART (Amplicor HCV Lys.V2; Roche Diagnostics); it was then reverse transcribed, and the V3 loop was amplified as described previously for episomal DNA with primers B1 and B2 (Titan One Step; Roche Diagnostics). The corresponding PCR products were purified, cloned, sequenced, and subjected to phylogenic analysis as described elsewhere.[6]

As shown in Figure 1, the sequences of double-LTR HIV DNA isolated during long-term effective HAART showed limited heterogeneity, arguing against a long-term archiving process, which requires high stability, as has been shown for the latent resting CD4 T-cell reservoir.[6] Diversity of episomal DNA sequences was lower compared with virus sequences from pre-HAART plasma, as shown by the mean genetic distance analysis (patient A: 0.23% ± 0.1 vs. 0.9% ± 0.5, patient B: 2.3% ± 1.6 vs. 5% ± 1.5, and patient C: 3.02% ± 1.6 vs. 4.3% ± 1.2, respectively). The pre-HAART plasma virus sequences and the double-LTR episomal sequences obtained during long-term effective HAART showed low heterogeneity in patient A, who started HAART early at a CD4 count of 550 cells/mm^3 (compared with 300 and 221 cells/mm^3 in patients B and C, respectively). It is also noteworthy that patient A took quadruple-drug therapy, whereas patients B and C took triple-drug therapy (see Fig. 1). More importantly, in the 3 patients, the episomal sequences were clearly distinct from the pre-HAART plasma virus sequences, indicating that episomal HIV DNA found during long-term optimal HAART does not result from archiving of pre-HAART replicating viruses.

The studies suggesting that episomal HIV DNA was highly stable were based on quantitative monitoring of episomal HIV DNA after cell infection in vitro,[4,5] and this may not accurately reflect conditions prevailing in vivo. Some authors have also measured HIV episomal DNA in patients' cells before and after HAART.[7] HAART was found to have little impact on the level of double-LTR HIV DNA, but this could also be explained by continual replenishment attributable to residual virus replication. Here, by examining HIV sequences in vivo, we found that double-LTR episomal DNA sequences during long-term effective HAART were distinct from pre-HAART sequences. *Env* sequences of PBMC provirus have been reported to evolve despite effective HAART, a phenomenon the authors attributed to residual virus replication.[8,9] This evolution might be better represented by HIV double-LTR circles than by total proviral DNA. Indeed, the latter includes integrated viruses from the latent reservoir, which we have shown to undergo long-term archiving.[6] Together, our data argue against the long-term stability of HIV double-LTR circles. These viral forms therefore should remain under consideration as potential markers of residual virus replication during HAART.

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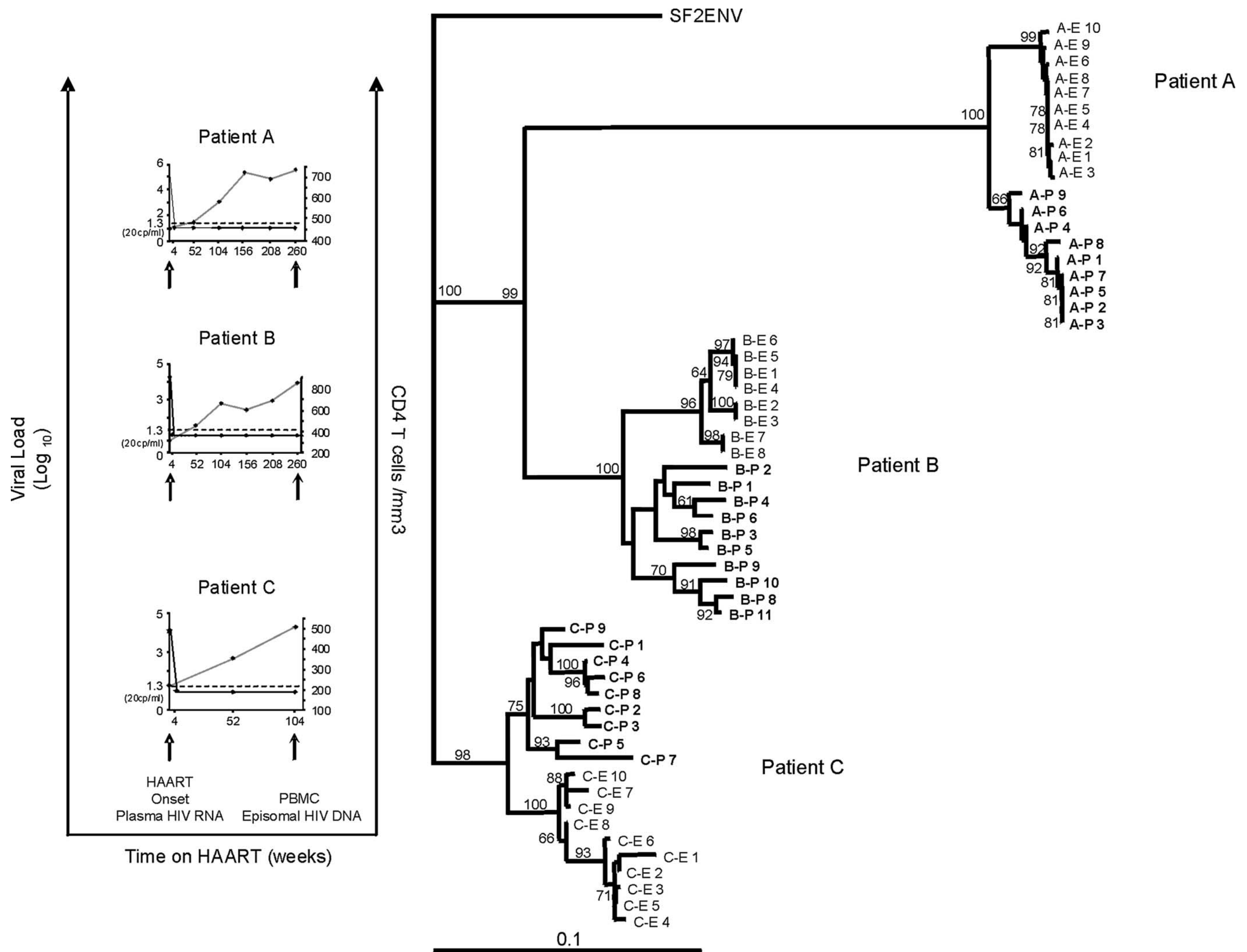

FIGURE 1. We selected 3 patients on long-term effective HAART for ≥2 years. Plasma HIV RNA was sampled 3 to 4 weeks before the outset of first-line HAART. The time course of the plasma viral load and the CD4 cell count during HAART is indicated, along with the PBMC sampling times for HIV double-LTR circle analysis. Phylogenetic analysis of V3 sequences of PBMC 2-LTR episomal HIV DNA (E) and those of pre-HAART plasma HIV RNA (P) is shown. Each clone is accompanied by the patient's designation (A–C) and the origin (E or P). The numbers at the internal nodes indicate the percentage of 100 bootstrap replicates that reproduced the clade.[10] Only values >60% are shown. The neighbor-joining tree is shown.[11] The reference sequence SF2 was used as an outgroup.

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The Effect of Lamivudine Therapy and M184V on the Antiretroviral Activity of Didanosine

To the Editor:

Initial antiretroviral therapy of HIV-1 infection frequently contains lamivudine (3TC) as one of the components. Individuals failing 3TC-containing therapy frequently have HIV-1 variants with a reverse transcriptase (RT) gene mutation (methionine-to-valine substitution at codon 184 or M184V) conveying high-level resistance to 3TC and modest in vitro cross-resistance to didanosine (ddI).[1] Despite this observation, the M184V mutation does not seem to decrease the antiretroviral response to ddI in vivo markedly,[2,3] although isolating the specific impact of M184V on the ddI response is difficult. ddI added to existing therapy provided better short-term virologic responses compared with placebo, despite the fact that most patients had baseline (BL) M184V in addition to many other nucleoside reverse transcriptase inhibitor (NRTI) mutations.[3] Few patients in this study lacked the M184V mutation, and, paradoxically, these individuals had poor responses. In addition, the relation between HIV-1 phenotypic susceptibility to ddI and the antiretroviral effect of ddI requires further clarification. In our study,[4] ddI was the only active antiretroviral given to patients with and without M184V and no or modest numbers of other NRTI mutations. Phenotypic susceptibility to ddI was assessed retrospectively, allowing us to dissect the effects of these factors on ddI activity in relative isolation and providing important information for clinicians assessing relative activity of ddI when used as a component of multidrug therapy in treatment-experienced patients.

Adult Clinical Trials Group (ACTG) 307 was a randomized, multicenter, double-blind study in which patients received ddI alone or in combination with hydroxyurea (HU) for the first 12 weeks of study.[4] Randomization was stratified based on prior antiretroviral treatment experience. Subjects on antiretroviral medications discontinued therapy for 14 days before enrollment. The study population for this retrospective analysis was defined as those subjects who were treated initially with ddI plus HU placebo or ddI plus active HU, had a week 8 HIV-1 RNA result on therapy, and had a BL HIV-1 RNA level greater than 400 copies/mL.

Using entry or pre-entry samples (27% and 73%, respectively), the HIV-1 RT region corresponding to codons 17 through 237 was sequenced using (Applied Biosystems, Foster City, CA) methods. Viral RNA was isolated from 140 μL of patient plasma with a QIAamp viral RNA kit (Qiagen, Valencia, CA). The RNA was eluted with 50 μL of RNAse-free water. Reverse transcription and polymerase chain reaction (PCR) amplification of 10 μL of the viral RNA solution were carried out using oligonucleotides derived from the HIV-1 subtype B pol gene consensus sequence. The sequence of the purified 665-base pair (bp) RT-PCR product was determined by Applied Biosystems PRISM BigDye Terminator cycle sequencing with AmpliTaq DNA polymerase, FS (Applied BioSystems) on an Applied Biosystems 377XL-96 DNA sequencer. The nucleotide sequences were aligned using Vector NTI (Informax, Carlsbad, CA) and compared with the sequence of the reference strain, HXB2. Sequences were manually confirmed and reviewed. Changes in HIV-1 RNA from BL to week 8 were examined in subjects grouped by 3TC experience and by the presence of M184V at BL. Mean change was estimated by parametric censored-data methods adjusted for BL viral load (VL) centered at 4.5 $\log_{10}$ copies/mL.[5] Analyses were also stratified by the ddI and ddI plus HU arms. Proportions with a week 8 HIV-1 RNA level <400 copies/mL were compared using the Fisher exact test. Phenotype assays for ddI susceptibility at BL were performed by Monogram Biosciences (South San Francisco, CA) with results reported as a fold-change (FC), which is the ratio of the 50% inhibitory concentration (IC_{50}) to ddI for the patient virus over the IC_{50} of the assay control wild-type HIV-1.

Of the 134 subjects enrolled in the randomized study, 75 received initial ddI-containing therapy (26 received ddI alone and 49 received ddI with HU) and had a BL HIV-1 RNA level >400 copies/mL and a week 8 result on initial therapy. Forty-three (57%) were antiretroviral experienced, and 24 (32%) had previous 3TC. The BL median HIV RNA level (VL) was 4.4 $\log_{10}$ copies/mL, and the BL median CD4 count was 388 cells/mm^3 and both were similar between 3TC-naive and -experienced patients. The BL RT sequence was available on 71 subjects. Nineteen had an M184V mutation; 10 of these had additional NRTI resistance mutations. Three had 2 accompanying thymidine analog mutations; the remainder had 1 or none.[6] Six BL samples had RT nucleoside resistance mutations but lacked the M184V. No samples had L74V or I, K65R, 151 complex, or 69 insertion mutations. Sixty-six patients had BL ddI phenotype results, with a median FC of 1.0 and a narrow range (0.7–1.9). The relations between treatment history (Fig. 1A), BL genotype (Fig. 1B), and ddI phenotype are shown in Figure 1.

In the week 8 analysis, including all patients initially treated with ddI or ddI plus HU, patients naive to 3TC had an approximately 0.4 $\log_{10}$ copies/mL greater mean decline in HIV-1 RNA than 3TC-experienced subjects (1.0 and 0.6 copies/mL, respectively, ddI arm; 2.0 and 1.6 copies/mL, respectively, ddI + HU arms; stratified $P = 0.06$). The mean decline in HIV-1 RNA level at week 8 in patients with M184V was less than in

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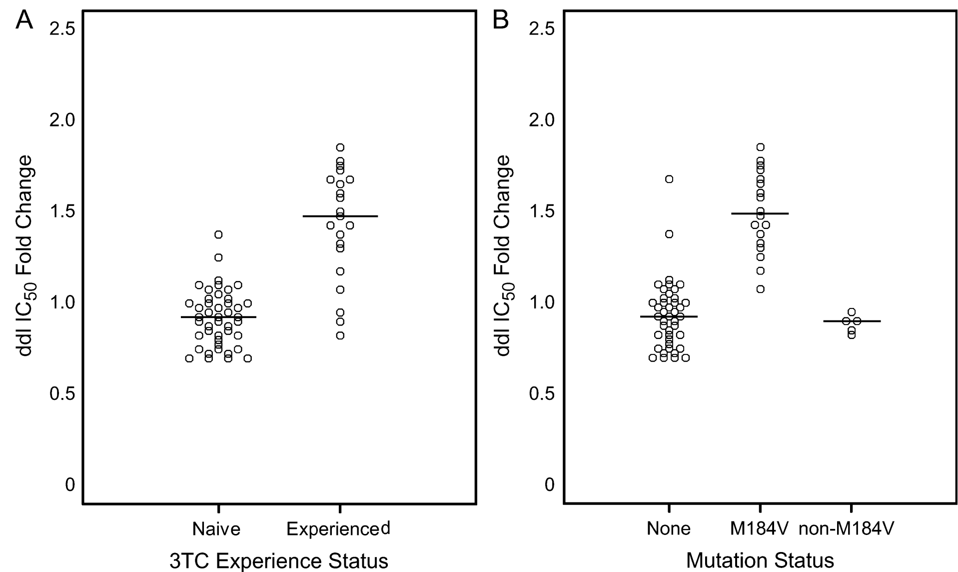

FIGURE 1. ddI phenotypic FC at BL in relation to 3TC treatment (A) and the presence of M184V or other non-M184V NRTI mutations (B). Horizontal bars denote medians.

those without M184V regardless of previous 3TC experience but not significantly so (stratified $P = 0.24$). For subjects with a BL genotype, 4 (21%) of 19 with M184V achieved an 8-week VL <400 copies/mL, whereas 22 (42%) of 52 of those without M184V had a VL load <400 copies/mL ($P = 0.16$).

VL response models accounting for censoring and including BL ddI FC and VL as covariates demonstrated a consistent relation between VL change and increasing FC, with a 0.7 $\log_{10}$ lesser decrease in VL for every unit increase in FC ($P = 0.06$). Results were similar when broken down by ddI and ddI plus HU treatment groups and when maximum VL response was the endpoint. The likelihood of attaining an 8-week VL <400 copies/mL diminished with increasing FC. The ddI FC values most strongly associated with an inflection in VL response using a maximum likelihood model were consistently in the range of 1.5 to 1.7 for pooled and subgroup analyses and using maximum VL response as the viral endpoint. Twenty-four (44%) of 55 subjects with a BL FC <1.5 achieved an 8-week VL <400 copies/mL, whereas only 1 (9%) of 11 subjects with a FC $\geq$1.5 had the same response ($P = 0.04$).

Our study is limited by the relatively small sample size, the censoring of VL data by the assay lower limit of 400 copies/mL, the relatively narrow IC_{50} range, and the fact that resistance tests were obtained a median of 7 days before entry; therefore, individuals previously on therapy were off therapy for a brief period before resistance testing. The fact that ddI was the only antiretroviral used in our study enhances our ability to interpret the results, however. Our results show that the M184V mutation clearly has an impact on ddI phenotype (see Fig. 1B). Consistent with the results of others,[2,3] we show that ddI has activity in the face of 3TC experience and in the presence of the M184V mutation, but these factors seem to diminish the antiviral effect of ddI. We also show that there is a consistent but narrow or compressed relation between ddI phenotype and antiretroviral response, with small increments in FC resulting in substantial reductions in the activity of ddI. Consistent with published lower and upper clinical cutoffs established for ddI in this assay (1.3- and 2.2-fold greater than controls),[7] we found an inflection point in ddI activity between an FC from 1.5 to 1.7 and only 1 subject with an FC $\geq$1.5 achieved a VL <400 copies/mL at week 8. Similar to other antiretrovirals, ddI has its greatest activity in patients with wild-type virus and low FC on phenotype. Although diminished, ddI has persistent activity in the presence of the M184V mutation whether in the setting of modest treatment experience such as subjects in our study[4] or in the face of more substantial treatment experience and nucleoside resistance mutations.[3]

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High Prevalence of Primary Antiretroviral Resistance Among HIV-1–Infected Adults and Children in Bahia, a Northeast State of Brazil

To the Editor:

The introduction of highly active antiretroviral therapy (HAART) for treatment of AIDS patients significantly decreases AIDS-associated morbidity and mortality.[1] Resistance to antiretroviral (ARV) drugs may arise during treatment, however, and is considered the main cause of therapy failure.[2] Although the major reason for the onset of drug resistance is poor adherence to treatment, acquisition of resistant strains of HIV also contributes to therapeutic failure.[3–5]

Brazil was the first country to provide free-of-cost ARV drugs for HIV therapy. This raised concerns about the selection and dissemination of drug-resistant HIV because of poor adherence and the extensive exposure to ARV drugs. Nevertheless, the Brazilian AIDS program is considered a success, and the available data show a low rate of HIV-1 drug resistance among drug-naive patients.[6]

To determine the prevalence of resistance mutations among recently diagnosed HIV-infected drug-naive patients, we evaluated plasma or HIV-1 RNA samples stored at the Retrovirology Laboratory of the Federal University of Bahia Hospital in Salvador, Brazil. The samples were obtained from the plasma of recently diagnosed patients from our AIDS clinics and from local blood banks after the year 2000. The institutional Ethics Review Board approved the study.

A total of 140 samples were tested for HIV resistance: 8 came from blood donors, and 132 came from our AIDS clinics (including 26 samples from vertically infected children). All samples were collected between 2000 and 2004 and were stored in a freezer at −80°C.

Resistant mutations were assessed in HIV-1 reverse transcriptase (RT) and protease (Pro) regions using the TruGene sequencing system (Visible Genetics, Toronto, Canada).

A multiple sequence alignment of the examined region and the related sequences in the GenBank/European Molecular Biology Laboratory (EMBL) database was performed with Clustal X software (available at http://ftp.ebi.oc.uk/pub/software/dos/clustalX) and further edited with the GeneDoc program (available at http://psc.edu/biomed/genedoc). The substitution model was selected with Modeltest 3.06 (available at http://darwin.uvigo.es/software/modeltest.html). Neighbor-joining (NJ) and maximum-likelihood (ML) phylogenetic analyses were performed using PAUP*4.0b10 (available at http://paup.csit.fsu.edu). The reliability of the NJ trees was evaluated by analyzing 1000 bootstrap replicates. A likelihood ratio test was used to calculate the statistical support for the branches (expressed in *P* values). The recombination analysis was performed using Simplot 3.5.1 (available at http://sray.med.som.jhml.edu/SCROftware/simplot).

To confirm the HIV-1 subtype assignment, we sequenced an *env* fragment of 34 samples that had enough remaining material for testing through reverse transcription of RNA samples using the enzyme Superscript III (Invitrogen, Carlsbad, CA). The resulting complementary DNA (cDNA) was subjected to nested polymerase chain reaction (PCR) amplification using V3 to V5 outer primers for the first round, with 3 options of primers for the second round. After sequencing the reaction, the samples were analyzed using the software Sequencing Analysis version 3.7 (Applied Biosystems, Foster City, CA) and Sequencher version 4.2 (available at http://sequencher.bio.indiana.edu).

We detected 39 different mutations associated with HIV resistance, totaling 307 amino acid substitutions. The most prevalent mutation was L63P (present in 48% of samples), whereas M36I was detected in 52 (39%) samples and L10V/I was found in 30 (23%) samples. Twenty-five (18.9%) samples presented at least 1 drug-resistant mutation: 15 samples (11.4%) had at least 1 mutation related to resistance to nonnucleoside reverse transcriptase inhibitors (NNRTIs), 13 (9.8%) to nucleoside reverse transcriptase inhibitors (NRTIs), and 7 (5%) to protease inhibitors (PIs). We found genotypic resistance to NRTIs and PIs in 3.8% of samples and to NRTIs and NNRTIs in 3.0%. Mutations associated with resistance to all NRTIs were detected in 6 (4.8%) samples, to all NNRTIs in 13 (9.8%) samples, and to all PIs in 3 (2.4%). Three patients presented resistance to all NRTIs and all PIs, preserving only NNRTIs as a therapeutic option. In addition, 2 other samples showed complete resistance to NRTIs and NNRTIs, leaving only PIs as active drugs.

Among the 26 children, we detected resistance mutations in 7 (26.9%): 5 presented with NNRTI resistance; 3 had genotypic evidence of resistance to NRTI; and 1 showed the mutation I50V in the Pro region, which is related to resistance to amprenavir. Two children showed resistance mutations to NRTIs and NNRTIs. We evaluated 1 mother-child pair: the mother's genotypic profile showed the mutations V75M, M184V, D30N, M36I, L63P, and N88D, but the analysis of the sample obtained from her baby revealed that she transmitted an HIV-1 strain harboring only the M184V mutation.

The phylogenetic analysis of the total *pol* region shows that of all the samples, 117 (84%) belong to subtype B with a low bootstrap value but highly significant ML ($P < 0.001$); 12 (8%) were

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subtype F and 3 (2.5%) were subtype C, both with $P < 0.001$ for ML. Twenty-one strains were classified as recombinants: 20 (14%) B/F and 1 (0.5%) B/C, with 61% and 89% bootstrap values, respectively ($P < 0.001$ for ML). Because this is the first time a study has found subtype C virus in Salvador, this may suggest a recent introduction of this subtype in the city. The presence of the 2 B/F recombinant clusters (69% and 100% bootstrap values and $P < 0.001$) suggests at least 2 distinct recombinant events. The bootscanning analysis of the B/F strains showed 2 different profiles of these 2 recombinant clusters, reinforcing that possibility.

Brazil has a much lower prevalence of HIV primary resistance than other developed countries do.[6] This provides a strong argument favoring the Brazilian policy of ARV drugs. Preliminary findings in Bahia have already raised some concerns about the real extent of drug resistance, however. A previous report detected 20% azidothymidine (AZT) resistance in peripheral blood mononuclear cells (PBMCs) of drug-naive subjects using a line probe assay (LiPA).[7]

The present work shows a worrisome scenario. The prevalence of primary genotypic drug resistance is much higher than previously estimated for Brazilian patients, and for the first time, we see evidence of resistance to more than 1 class of ARV drugs in drug-naive patients. The high prevalence of resistance to NNRTIs in our study is in accordance with what is generally seen in other parts of the world.[8,9] This is a likely consequence of the wide use of these drugs in first-line regimens and the tendency of NNRTI mutations to persist because they do not significantly compromise the replicative capacity of the virus.[10] Also, the known low genetic barrier of that class of drugs contributes to the easier selection of resistance in a setting of nonstrategic interruption of treatment. The high frequency of resistance to other drug classes was surprising, however.

The high rate of genotypic resistance among drug-naive children vertically infected was also unexpected. The Brazilian guideline still accepts prophylaxis with AZT or AZT plus lamivudine (3TC) for pregnant women who do not fulfill the criteria for ARV treatment. Our results probably reflect the late diagnosis of HIV infection for most mothers, absence of proper prophylaxis against vertical transmission, and poor adherence among those who used ARV drugs but did not avoid mother-to-child transmission.

The analysis of viral subtypes provides additional evidence supporting the previous report on the circulation of non-B subtypes in Brazil. Despite the fact that only one third of our samples could be sequenced for subtype confirmation, the finding of 12 subtype F, 3 subtype C, and 21 recombinant forms confirms the high genetic diversity of HIV-1 in this region. The finding of an almost complete resistance profile in 1 patient harboring a subtype F strain is intriguing. It has been reported that in non-B subtypes, resistance to specific ARV drugs could evolve along different pathways than in B strains.[11] This subtype F case showed complete resistance to all but NNRTI drugs, raising the possibility that multidrug-resistant non-B subtypes are being transmitted.

The present report indicates that HIV primary resistance in Brazil may be underestimated and suggests the need for evaluating specific populations. Our findings reinforce the recent decision from the Brazilian Ministry of Health making mandatory the perinatal screening for HIV-1 antibodies among women not tested during pregnancy, along with resistance testing for those women with a viral load greater than 5000 RNA copies/mL. Resistance testing for children of HIV-infected mothers may be required to avoid future therapy failure because of transmitted HIV-1–resistant strains. The high prevalence of HIV-1 B/F recombinant strains in this population is also of note. Given these peculiarities, monitoring the genetic diversity of HIV-1 is probably important for the development of vaccines and treatment strategies.

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Investigating Iatrogenic HIV Transmission in Ugandan Children

To the Editor:

Biraro and colleagues[1] present data on childhood HIV infections, health care exposures, and maternal serostatus in a rural Ugandan cohort. Their report focuses on 26 infected children younger than the age of 13 years. Although there are data on transfusions and injections for nearly all these children, there are only 8 child-mother pairs with direct serostatus data on both the child and the mother (including 1 [13%] in which the mother tested seronegative after the child tested seropositive). For the other 18 children, the authors posited the mother's serostatus at the time of the child's seroconversion from such indirect evidence as the father's antiretroviral treatment up to 7 years after the child's birth. From these combined indicators, the authors conclude that at least 90% of the children acquired HIV vertically.

Their report rests on several assumptions that may lead to an overestimation of vertical transmission. First, they assume that infected child-mother pairs had linked infections. Although this is likely for most pairs, it is impossible to know without sequencing HIV isolates from the child and mother in each pair. If the authors had stored blood specimens, the degree of genetic HIV concordance could be examined. Second, in 1 definite instance and in all the probable instances of infected mothers who had infected children, the direction of transmission—if infections were linked—cannot be determined with the data presented. Child-to-mother transmission through breast-feeding has been suggested in several studies,[2–4] but the authors discount this possibility in their cohort by assuming that few children had acquired their infections iatrogenically.

The authors' own data suggest that iatrogenic transmission may have been significant, however. The HIV prevalence in their sample does not decrease as the children's age increases from 3 to 12 years of age (their Fig. 1), as would be expected if vertical acquisition predominates among infected children, and few vertically infected children survive to middle or late childhood. Furthermore, the stable HIV prevalence across this age range is not a cohort effect of declining prevalence over time, because the prevalences in children and women of reproductive age were virtually the same in this cohort 10 years earlier.[5,6] Flat prevalence curves across childhood age groups have been repeatedly observed in sub-Saharan Africa, even during expanding epidemics.[7–9]

Other research also points to more horizontal HIV transmission in Ugandan children than that allowed by Biraro and colleagues.[1] In the 2004 to 2005 Uganda Demographic and Health Survey (DHS),[10] based on a nationally representative sample, approximately 16% of infected children younger than the age of 6 years had seronegative mothers. This rate, like that constructed by the authors, represents a lower bound estimate for horizontal transmission. Moreover, in a national sample of Ugandan children with malaria,[11] approximately 2% of those younger than the age of 5 years and approximately 3% of those aged 5 to 9 years were HIV infected, which are much higher prevalences than the 0.7% in the DHS[10] or the 0.4% in the authors' sample. Malaria treatment, often involving multiple injections and transfusions, has been a major risk for HIV acquisition in children since early in the epidemic.[12] Transmission between mother and child—in either direction—not only can occur through breast-feeding but iatrogenically as well. A household survey in a district adjacent to Masaka conducted at the time many of the children in the authors' Masaka cohort were infected showed that 83% of households kept injection equipment at home, which was typically reused by multiple family members when visiting health care providers for treatment.[13,14] Such shared blood exposures could result in genetically linked infections within households, such as between mothers and children. Still other blood exposures (eg, traditional surgery,[15] dental care,[15,16] incisions for administering herbal medicines, scarification) may be HIV transmission risks for children in Uganda but apparently were not assessed by the authors.

Further analysis of the data of Biraro and colleagues[1] may provide additional insight into the routes of transmission among children in their cohort. The authors presented results for children tested in round 11 only, although similar data on the serostatus of mothers and children and blood exposures have been analyzed for rounds several years earlier.[5,17] All available previously unanalyzed data from children in the cohort should be reported for a complete accounting, if only to note the number and proportion of infected children who had seronegative mothers at the time they seroconverted. Curiously, the authors group children who had no injections with those who had up to 5 therapeutic injections in the past year and those who had up to 4 vaccination injections (their Table 4). It is crucial to report results for unexposed children compared with those with varying degrees of exposure to therapeutic injections, vaccination injections, and injections of either type. Childhood vaccination (in presumably unhygienic conditions) has been associated with HIV infection in Guinea-Bissau[18,19] and South Africa.[16] The 1 child with incident HIV infection in a previous report on the Masaka cohort was a breast-feeding girl who seroconverted in the same round as her mother and, notably, had received vaccination injections.[17] A thorough updated assessment of incident infections in children in the cohort would also be valuable. Although the association between health care exposures and HIV could reflect treatment for HIV-related symptoms, the available empiric evidence suggests that this may not account for observed relationship in adolescents and adults.[20,21]

HIV incidence[17] and prevalence[1,5] in children are markedly lower in Masaka than in many other parts of sub-Saharan Africa,[7–9,16] and this may be partly attributable to greater awareness in Uganda of the risk of iatrogenic infection.[13,22] Nonetheless, the routes of transmission among children in Uganda and elsewhere in sub-Saharan Africa should be investigated more comprehensively and intensively than heretofore.

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Response to "Factors Associated With Self-Efficacy for Condom Use and Sexual Negotiation Among South African Youth"

To the Editor:

In their article, Sayles and colleagues[1] addressed meaningful information on "Factors Associated With Self-Efficacy for Condom Use and Sexual Negotiation Among South African Youth". South Africa is one of the most affected countries in the AIDS epidemic.

The authors adopted a self-cognitive model to identify variables that could enable elucidation of the pattern of self-efficacy for condom use and sexual negotiation. They ignore the variables in the model in the multiple logistic regression, however. The modeling was based on adjustment for sociodemographic confounding factors and was performed as if the variables in the domains were not affected by those on other domains. We would expect a conceptual model to discuss the relation among variables[2] and take them into the multivariate analysis.

Regarding the self-efficacy for condom use and sexual negotiation, the authors used a 5-item scale, constructed in part from a previously validated 14-item scale of condom use self-efficacy, which had a 0.85 Cronbach α coefficient.[3] Nevertheless, no information was given about the validation process of the short scale. Considering the substantial decline of the Cronbach coefficient of the new scale, 0.64 for women and 0.60 for men, we anticipate that said scales have different constructs. Because α values higher than 0.7 are required for satisfactory comparisons between groups,[4] this paper has a relevant drawback that the authors failed to address in the discussion.

The use of a nonvalidated scale and the lack of adjustments for other confounding factors might explain why some known associations were not confirmed in this study. For instance, the authors did not find an association between HIV infection and the first sexual experience before 15 years of age, contrary to other studies[5,6] and our own findings.[7]

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Authors' Response to Letter to the Editor Regarding "Factors Associated With Self-Efficacy for Condom Use and Sexual Negotiation Among South African Youth"

Reply:

Alencastro et al raise concern about the inclusion of appropriate variables in the multivariate model, which includes the outcome of self-efficacy in this analysis. We would like to clarify that the multivariate model as displayed in Tables 3 and 4 of the paper included all candidate variables that had been previously identified in each domain of the conceptual model, in addition to sociodemographic characteristics. We agree with Alencastro et al that it is important to take into account the relation between variables included in a conceptual model within the context of a multivariate analysis. To address this in our study, collinearity was examined between independent variables and we tested for interactions that we hypothesized may exist in our model. No significant collinearity or interactions between covariates were identified. Finally, a sensitivity analysis was performed and showed that the significant factors associated with the outcome of self-efficacy were stable and did not change when other covariates were included or omitted from the final model. The details of these additional analyses were not included in the original paper for clarity of presentation and because they were not found to have a significant impact on the analysis.

With regard to the self-efficacy for condom use and sexual negotiation index, we agree with Alencastro et al that the ideal measure would be one whose validity has been tested among South African youth. Such a measurement was not available at the time the National Youth Survey was conducted, however. The self-efficacy index that was used in our analysis was derived from an often used measure with proven reliability and high validity among US adolescents and youth in the developing world.[1,2] Thus, we attempted to include a shorter version of the best measure available to us at the time of the survey. The intermediate coefficient α of our scale suggests that our 5-item index may not be as sensitive a measure of self-efficacy in South African youth. This means that we may be underestimating the magnitude of relations between key factors and self-efficacy—in effect, biasing our results toward the null. In this context, relations between factors in our conceptual model and self-efficacy may be stronger than we were able to detect in this analysis. Nevertheless, this measure was strongly associated with condom use at last sex[3] and condom use consistency[4] in these data. Future work on a valid and reliable measure of self-efficacy for condom use and sexual negotiation among South African youth is needed to characterize these relations better.

Finally, we would like to clarify that the goal of this particular analysis was not to examine the association between HIV infection and other factors such as first sexual experience under the age of 15 years, for which Alencastro et al reference 3 studies. The focus of the paper was on identifying factors associated with self-efficacy for condom use and sexual negotiation. Any limitations in measurement of self-efficacy certainly would not apply to the lack of finding a significant relation between HIV infection and early age of sexual initiation in the overall results of the National Youth Survey, as is incorrectly suggested by Alencastro et al. This paper did find a number of factors to be significantly associated with self-efficacy for condom use and sexual negotiation, and this information may prove to be beneficial for individuals designing HIV prevention programs among youth in South Africa.

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