In human immunodeficiency virus type 1 (HIV-1)–infected persons, virus load (serum/plasma level of HIV) predicts outcome. Virus load trends have been characterized in adults and infants but not in children. Virus load trends in 22 male children with hemophilia who acquired HIV-1 postnatally (age 0.7–5.2 years at seroconversion) were studied. The mean HIV-1 load 2 years after seroconversion was 4.40 log_{10} copies/mL, and the mean change over time (slope) was 0.03 log_{10} copies/(mL·year). Significant among-children variation was apparent: a random effects model predicted that 95% of children had early virus loads 3.75–5.04 log_{10} copies/mL and slopes −0.07 to 0.12 log_{10} copies/(mL·year). Higher early virus loads and higher slopes were each associated with increased mortality (P = .006 and P = .03, respectively). In conclusion, those subjects had virus load trends similar to those in adults. Early virus loads were lower than those in vertically infected infants, which suggests that factors changing soon after birth affect viral replication.

Subjects and Methods

Subjects and clinical outcomes. The Multicenter Hemophilia Cohort Study (MHCS) is a prospective cohort study of hemophilia subjects enrolled at 16 treatment sites in the United States and Europe, beginning in 1984 [9]. Enrolled subjects are seen every 6–12 months. HIV-1 seroconversion dates previously were estimated by using individuals’ HIV-1 test results and data on use of clotting factors [10]. For this study, we included MHCS subjects who were HIV-1 infected by clotting factor products at age <6 years. Included subjects had available for virus load testing ≥2 stored serum or plasma samples obtained ≥1 year apart. In addition, the first available sample for included subjects had been obtained within 2.5 years after seroconversion.

Laboratory measurements. Plasma or serum samples were separated from whole blood and were frozen on the same day (2–8 h after sampling) or after overnight shipment. Specimens were stored at −70°C until tested. We measured virus loads (Amplicor
HIV Monitor assay; Roche Molecular Systems) in the first available sample after HIV-1 seroconversion and subsequent samples ~1 year apart in time. Heparin, a potential inhibitor, was removed from plasma samples by use of silica extraction. A value of 100 copies/mL was assigned to readings below the assay’s threshold of 200 copies/mL, and results obtained from serum were multiplied by 1.32 to make them comparable with plasma values [7].

For each subject, we analyzed all available measurements of the percentage of lymphocytes that were CD4 positive (CD4%). We used CD4% values instead of absolute CD4 lymphocyte counts, because the total lymphocyte count decreases substantially during normal childhood. Measurements were performed on mononucleated stained lymphocytes, using flow cytometry.

Statistical methods. For descriptive purposes, we fitted time trends to each subject’s log10-transformed virus load, using linear regression (“observed” trends). This approach is convenient, but it inappropriately assumes that, apart from the time trend, successive observations for any individual are uncorrelated. We fitted a random-effects model to study virus load trends more formally [11]. Under this model, the log10 virus load for subject i at year t_{ij} (denoted Y_{ij}) was modeled as Y_{ij} = \beta_0 + \beta_1(t_{ij} - 2) + e_{ij}, where \beta_0 was the i-th subject’s early log10 virus load and \beta_1 was the i-th subject’s virus load slope. This model was centered so that \beta_0 values were the expected log10 virus loads at 2 years after seroconversion. The early virus loads (\beta_0 values) and slopes (\beta_1 values) could vary among individuals and were each considered to be normally distributed. For each individual i, we modeled the serial correlation among the errors e_{ij} with a negative exponential function [11].

Using the random-effects model, we estimated the population means for early virus load and slope, which provided an estimate of the average trend. We also assessed whether individuals differed in their time trends by testing whether the variance among individuals in early virus loads or slopes was significantly greater than zero. In addition, we evaluated models with terms for age at HIV-1 seroconversion and antiretroviral therapy at time t_{ij} (any vs. none).

The random-effects model gave estimates for each child’s early virus load and slope. These estimates for each individual (the separate estimates for \beta_0 and \beta_1) were each weighted averages of that individual’s observed values and the population means, so that the estimates were pulled toward the means.

We performed similar modeling for CD4% measurements. To examine the relationship between early viral replication and subsequent loss of CD4 lymphocytes, we calculated the Pearson correlation coefficient between random-effects estimates of early virus loads and CD4% slopes. In other analyses, we used the Wilcoxon rank sum and log-rank tests to compare continuous outcomes and incidence rates, respectively, between groups. Results were considered to be statistically significant (P < .05) or borderline significant (P = .06–.10).

Results

Study subjects. Eighty-nine children had HIV-1 seroconverted at age <6 years. Of these 89 children, 22 (25%) had sufficient serial serum or plasma samples for inclusion in the present study (most excluded subjects lacked samples within 2.5 years of seroconversion). For the 22 children who were included (all males), the median age at seroconversion was 3.4 years (range, 0.7–5.2 years). Included subjects seroconverted between 1981 and 1985. During 270 person-years after seroconversion, 6 children developed AIDS-associated opportunistic illnesses, and 5 died. Compared with children in the study, excluded children had similar ages at seroconversion (P = .95), incidence of opportunistic illness (P = .74), and mortality (P = .38).

Virus load measurements. Included children had 150 virus load measurements (median, 6 measurements/subject; range, 4–10 measurements/subject; median, 1.4 years between measurements), with initial measurements at a median of 2.0 years after seroconversion (interquartile range, 1.0–2.2). Figure 1 displays virus load measurements for 10 representative subjects and “observed” time trends fitted with linear regression. These trends fitted most subjects’ measurements well, although substantial variation was apparent for some subjects (e.g., subjects E and G).

Under a random-effects model, the mean early virus load was 4.40 log_{10} copies/mL (95% confidence interval [CI], 4.14–4.66) and the mean virus load slope was 0.03 log_{10} copies/(mL·year) (95% CI, −0.02 to 0.07). The population mean trend derived from these values also is shown in figure 1. Individual observed trends varied markedly around this mean trend (figure 1). Indeed, variances among subjects in early virus loads and slopes were each significantly greater than zero when considered separately (P = .05 for each), and together they were borderline significant (P = .07), which implies the presence of true differences among subjects. The model estimated that 95% of individuals had early virus loads between 3.75 and 5.04 log_{10} copies/mL and virus load slopes between −0.07 and 0.12 log_{10} copies/(mL·year).

The random-effects model also provided separate estimates for each individual’s virus load trend (figure 1). Observed and random-effects trends agreed for most subjects. However, for a few subjects the observed early virus loads or slopes appeared to be extreme (subjects B and J), and their random-effects trends were pulled toward the population mean.

Age at seroconversion was not significantly related to early virus load or slope (P = .37 and P = .18, respectively). Subjects were receiving antiretroviral therapy at 30 time points (20%), most of which was monotherapy (23 time points; 77%). Virus load was not related to antiretroviral therapy (mean virus load, 4.70 log_{10} copies/mL with therapy vs. 4.46 log_{10} copies/mL without therapy; P = .58).

As shown in figure 2A and 2B, high early virus load and high virus load slope were each significantly associated with increased mortality.

CD4% measurements. There were 334 CD4% measurements (median 17 measurements/subject; range, 2–32 measurements/subject; median, 0.5 years between measurements). Under a random-effects model, the mean early CD4% (2 years after seroconversion) was 31% (95% CI, 28%–34%), and the mean CD4% slope was −1.7% per year (95% CI, −2.3% to −1.2%). Under this model, all children had the same early CD4% (namely, 31%); however, uncertainty in this prediction
Figure 1. Virus load measurements for 10 representative human immunodeficiency type 1 (HIV-1)–infected children. For each subject, serial virus load observations (log10 copies/mL) are indicated by points connected by solid black lines. For each subject, the single solid black line drawn across all observations indicates the time trend derived by using linear regression (referred to in the text as the “observed” time trend). The dotted line indicates the time trend fitted by using the random-effects model, and the gray line indicates the population average trend (same in each panel). The vertical dashed line, at 2 years after seroconversion, marks the time for which early virus loads were estimated. Subjects are ordered by their age at HIV-1 seroconversion, which is noted at the bottom left of each panel.

Discussion

In those children who acquired HIV-1 infection through clotting factor infusions, HIV-1 load trends resembled trends previously reported for adults [2, 6, 12]. We found that the mean virus load was 4.40 log10 copies/mL 2 years after seroconversion. In comparison, for MHCS subjects who HIV-1–serocon-
Figure 2. Survival of human immunodeficiency virus type 1 (HIV-1)–infected children as a function of random-effects estimates for early virus load (A) or virus load slope (B). In each panel, Kaplan-Meier plots are shown, with subjects divided at the median value (4.40 log10 copies/mL for early virus load; 0.03 log10 copies/[mL·year] for virus load slope). In each panel, the solid line corresponds to children with values below the median, and the dashed line corresponds to children with values above the median. The difference in survival between the 2 groups is significant in both analyses (P values shown).

verted at 35–70 years old, the median virus load 12–36 months after seroconversion was 4.08 log10 copies/mL [2]. After this early period, virus loads in our subjects tended to increase slowly (mean slope, 0.03 log10 copies/[mL·year]). This increase was similar to, but slightly more gradual than, increases reported for adults (0.03–0.09 log10 copies/[mL·year]) [6, 7, 12].

In contrast with these children and with adults, perinatally infected children have higher virus loads early in infection (typically averaging 5.00–5.20 log10 copies/mL at 24–36 months of age), and their virus loads tend to decline subsequently [4, 5, 8]. These differences suggest that critical changes occur in the first year of life that can affect early viral replication. Cell-mediated immunity may be important in this regard [13]. This system is immature at birth but, by age 1–5 years, may be able to control early viremia. Also, in fetuses and young infants, HIV-1 readily infects the thymus, which might augment plasma levels of HIV-1 [14]. Thymic involution begins after the neonatal period and could lead to lower virus loads with later infection. Similarly, absolute levels of CD4 lymphocytes are high in infancy, which could facilitate viral replication. We could not distinguish among these possibilities in our study.

High early virus load and steeply positive slopes were associated with elevated mortality. We also found an inverse, though nonsignificant, relationship between early virus load and CD4% slope. HIV-1–mediated destruction of CD4 lymphocytes may partly account for the deleterious effects of high HIV-1 load [1]. Nonetheless, HIV-1 replication can increase risk for disease progression independently of its effects on CD4 lymphocyte levels [3, 15].

We noted significant variation among children in virus load trends. For example, some children had positive slopes, and others had negative slopes. Possible reasons for this variation include qualitative or quantitative differences in the virus inoculated through contaminated factor infusions or genetic differences among children. Because individual trends truly differ among children, the population average trend is only partly informative.

For subjects who had few data or extreme observed trends, the random effects estimates were pulled toward the population average. In this way, our random effects model appeared to correct potentially unreliable observations. Notably, random effects estimates of early virus load and slope strongly predicted mortality, which suggests that the model was reasonable. These relationships were less apparent when observed trends were used instead (data not shown).

Our study is unique in its description of individuals who acquired HIV-1 infection in early childhood. In light of the difficulty in identifying informative subjects, its main limitation is the small number of children studied. Included children may have differed from excluded children, although we found no differences in age at seroconversion, incidence of opportunistic illnesses, or mortality. Our study included only male children, so our results conceivably might not apply to females. Also, because we had few virus load measurements within the first year after seroconversion, we were unable to characterize virus load patterns during...
primary infection. Finally, we had few data on combination antiretroviral therapy, because most follow-up occurred before 1996. Nonetheless, we did not see an effect of less effective therapy (mostly monotherapy) on virus loads, and our study design allowed us to observe biologically relevant trends that would have been obscured by more effective therapy.

In conclusion, HIV-1 load patterns in children with hemophilia more closely resemble patterns in adults than those in vertically infected infants. Among these children, as in other groups, HIV-1 loads provide prognostic information. Differences in trends among children justify continued attempts to identify host and viral determinants of disease progression.

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References