CLEARANCE OF HIV INFECTION IN A PERINATALLY INFECTED INFANT

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Abstract Background. We describe a child who was identified shortly after birth as infected with the human immunodeficiency virus type 1 (HIV-1), but whose infection appears to have completely cleared. Asymptomatic HIV-1 infection was diagnosed in the mother during the fourth month of pregnancy. The infant was delivered vaginally at 36 weeks, received no blood products, and was not breast-fed.

Methods and Results. HIV-1 was detected by culture of the infant's peripheral-blood mononuclear cells at 19 and 51 days of age. Plasma from the infant was also culture-positive for HIV-1 at 51 days of age by DNA polymerase chain reaction (PCR). Nucleotide-sequence analysis of HIV-1 DNA showed extremely close homology of the cultures obtained 32 days apart, and forensic markers of genetic identity for the two cultures were identical. Hence, inadvertent viral contamination or error in the collection of specimens was highly unlikely. At 12 months of age the infant was seronegative for HIV-1, and numerous subsequent cultures and tests by PCR have also been negative for HIV-1. The child is five years of age at this writing, is HIV-seronegative, and remains well, with normal growth and development and no laboratory or clinical evidence of HIV-1 infection.

Conclusions. The infant we describe was infected perinatally with HIV-1, but the infection subsequently cleared and the infant remained without detectable HIV-1 infection five years later. (N Engl J Med 1995;332:833-8.)

During the early stages of primary infection with the human immunodeficiency virus type 1 (HIV-1), viral titers in plasma reach high levels. This phase of viremia is followed by a rapid decline, in which viral titers in serum and the load of provirus in peripheral-blood lymphocytes decrease by several orders of magnitude, presumably as a result of cellular or humoral immune mechanisms.1 Lower levels of virus and viral DNA can then persist in asymptomatic people for 10 to 15 years.2 However, humans have not been known to have complete clearance of HIV-1.

It has been estimated that 13 to 40 percent of HIV-1-infected mothers transmit the virus to their infants,3 and most infected infants have symptoms of the acquired immunodeficiency syndrome (AIDS) within a few years. It has been difficult to investigate the early stages of HIV-1 infection in infants, since maternal HIV-1 IgG antibody persists in infants for up to 15 months. Thus, it has been necessary to measure HIV-1 directly by either culture for the virus or the polymerase chain reaction (PCR) for HIV-1 DNA sequences. Using these direct approaches in the early stages of HIV-1 infection in infants, we found several rare cases in which the infants had initial evidence of HIV-1 infection but in which the infections apparently cleared within a few months. In this report we describe one such case in detail.

Case Report

The infant was a 2.3-kg (4-lb, 11-oz) boy born at 36 weeks' gestation by normal vaginal delivery to a 33-year-old woman (gravida 4, para 2, abortus 2) who had been given a diagnosis of asymptomatic HIV-1 infection by routine prenatal testing in approximately the fourth month of pregnancy. The mother reported no intravenous drug abuse, but during the first few months of gestation she had had sexual relations with a former intravenous drug user. The pregnancy was uncomplicated. The mother had a normal absolute CD4 count (>1000 CD4 cells per cubic millimeter) during pregnancy and remained asymptomatic during the next four years.

The infant was normal at birth, but he required hospitalization for mild respiratory distress syndrome for the first eight days. He did not receive blood products or plasma and was not breast-fed. He was followed frequently for evidence of HIV-1 infection as part of a prospective study for early HIV-1 diagnosis. The infant underwent frequent physical examinations and remained asymptomatic subsequently, without any of the physical findings associated with HIV infection. His growth and development were normal, including normal neuro-psychological examinations for his age. At this writing he is five years of age and attending kindergarten. The results of his standard laboratory evaluations, including complete blood counts, analyses of blood chemistry, and measurements of serum immunoglobulins, CD4 T-cell subgroups, and CD4/CD8 ratios, have been normal. The re-
sults of laboratory studies of the infant, including HIV cocultures; measurements of plasma viremia, p24 antigen (Abbott, Abbott Park, Ill.), and acid-disassociated, immune-complex–dissociated (ICD) p24 antigen (Coulter, Miami); and serum HIV IgA antibody tests by enzyme-linked immunosorbent assay (ELISA) and Western blotting, are shown in Table 1.

Blood obtained from the baby at birth and 19, 33, and 51 days after birth was cultured for HIV-1 in peripheral-blood lymphocytes, as well as plasma, according to standard AIDS Clinical Trials Group (ACTG) methods (Table 1). The cultures of peripheral-blood mononuclear cells were negative at birth, positive 19 days after birth, negative at 33 days, and positive at 51 days of age with a relatively low titer (10^6 peripheral-blood lymphocytes). The quantitative plasma culture at 51 days was also positive at 100 median tissue-culture infective doses per milliliter. The assays of plasma p24 antigen and acid-disassociated, immune-complex–dissociated p24 antigen were negative in the infant, as were four HIV-1 IgA dot blot assays during the same period. The infant was clinically well and became negative for HIV antibody by ELISA and the Western blot assay at the age of 1 year, at 13 months an HIV-1 culture was again initiated and was negative. Numerous subsequent cocultures using up to 10^7 peripheral-blood lymphocytes from the infant were also negative for HIV-1. Many additional PCRs of the infant’s peripheral-blood lymphocytes have remained negative to the present time, five years after birth. The ELISA for HIV antibody remains negative, and the child remains asymptomatic.

**METHODS**

Blood samples were obtained from the baby at birth and 19, 33, and 51 days after birth, and at various times thereafter until he was five years old. After isolation, peripheral-blood mononuclear cells were cocultured with peripheral-blood mononuclear cells from healthy donors, and the titer of HIV-1 gag p24 antigen was monitored twice a week according to standard ACTG procedures. Quantitative plasma cocultures were also performed according to standard ACTG procedures at 33 and 51 days and at 3 months. DNA was isolated from the HIV-1 cultures of peripheral-blood mononuclear cells obtained from the infant at days 19 and 51 and was isolated directly from peripheral-blood mononuclear cells obtained from the mother 12 months after delivery by methods described previously. Briefly, the peripheral-blood mononuclear cells from the mother and the cultured samples from the infant were treated with phenol four or five times to remove proteins, then were extracted twice with chloroform–isoamyl alcohol, and were precipitated in ethyl alcohol. The isolated DNA was resuspended in TRIS-EDTA buffer (10 µM TRIS and 1 µM EDTA; pH 8). Quantitative PCR was performed to determine the concentration of HIV-1 in the samples. With labeled primers 5′M667 and 5′M661, 1 µg of DNA was subjected to 25 cycles of PCR. DNA with an equivalent of 200 copies of HIV-1 was used to ensure adequate representation of variant sequences. In later clinical samples of peripheral-blood lymphocytes from the child, primers SK38 and 39 were also used to confirm a negative PCR.

The 3′ primer, envi22 (GTGAGGGGAAATTCTTCTACTGTAA), and the 3′ primer, env21 (GGTACCACACATCTTGGTTAATTAG), were used for the PCR amplification and sequence analysis. PCR was performed in a buffer containing Taq polymerase (Boehringer, Mannheim, Germany). After 40 cycles of amplification, the PCR products were loaded onto a 5 percent polyacrylamide gel. Bands of 240 base pairs were recovered by soaking the gel overnight in 0.3 M sodium acetate, 10 mM TRIS–hydrochloric acid, and 1 mM EDTA. Negative control DNA isolated from HIV-1–negative donors and positive control DNA (10 to 1000 copies of the cloned JRCSF strain of HIV-1) were used to check for possible contamination and efficiency of amplification; there was no contamination.

The DNA recovered from the polyacrylamide gel was digested with EcoRI and ligated to the M13mp18 vector digested with EcoRI and Smal. The ligated DNA was introduced into XLI-Blue cells (Strategen, La Jolla, Calif.). Positive plaques selected by hybridization screening were sequenced with DNA sequencing kits (United States Biochemical, Cleveland). Sequences from the baby, the mother, and the HIV Database were assessed with the Clustal program. The nucleic acid sequences isolated from the mother and the infant have been deposited in the GenBank data base (accession numbers L30545 through L30574).

Sequences were obtained from three HIV-1 isolates from infants born in California during the same period as the study infant; HIV infection was diagnosed in all three. The sequences were cloned in the laboratory of one of the study authors and included in the comparison. The HIV envelope regions of these isolates (B1VC1, B2VC3, and B3VC14) were cloned into pGEM vector (Promega, Madison, Wis.) after amplification with outer primers 5′RedE1 (GTGACG-ACAGTCAATGTA; positions 6953 to 6972 of HIVBEC1) and 5′RedE2 (GCACACACTTCTCTTGCC; positions 7711 to 7730 of HIVBEC1) with inner primers 5′RedE3 (AATTCTTAGATTG-CCACGATGTAACA; positions 6982 to 7001 of HIVBEC1) and 3′RedE4 (AATTTAGCTTCCATGATGTCCCT; positions 7636 to 7657 of HIVBEC1) and digestion with XbaI and HinfIII. The V4 envelope regions of these clones were sequenced with primer RedE4 and automated fluorescent sequencing (Applied Biosystems,

Table 1. Laboratory Data on the Mother and the Infant before and after Birth.*

<table>
<thead>
<tr>
<th>Test</th>
<th>Mother</th>
<th>Infant</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV ELISA</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>HIV-1 culture PBL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Negative</td>
<td>Positive (10^6 PBL)</td>
</tr>
<tr>
<td>33</td>
<td>Negative</td>
<td>Positive (10^6 PBL)</td>
</tr>
<tr>
<td>51</td>
<td>Negative</td>
<td>Positive (100 TCID&lt;sub&gt;50&lt;/sub&gt;)</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>12–13</td>
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<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>28</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>36–60</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Dashes indicate not determined. PBL denotes peripheral-blood lymphocytes, TCID<sub>50</sub> median tissue-culture infective doses, ICD immune-complex–dissociated, and ELISA enzyme-linked immunosorbent assay.

†This test was repeated numerous times during this period.
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Foster City, Calif.). In addition, known sequences from 27 North American HIV isolates, the BRU and OYI isolates from western Europe, and the JRFL isolate from Zaire were used for comparison.

Genetic Typing of Samples

Additional aliquots of frozen peripheral-blood lymphocytes saved from the sample obtained from the infant on day 19, which was positive for HIV by culture, and those obtained at the age of 2½ years, when he was seronegative, as well as samples from the mother, were examined for genetic identity with the AmplicType PM PCR Amplification and Typing Kit (Perkin–Elmer Roche, Branchburg, N.J.). This kit includes reagents that allow the simultaneous amplification of specific regions of six genetic loci and the subsequent detection of their allelic variants. These independently segregating loci include HLA-DQA1 (DQA1), low-density lipoprotein receptor (LDLR), glycopherin A (GYPA), hemoglobin G gammaglobin (HBGG), D7S8, and group-specific component (GC). The combined power of discrimination for these markers for white people in the United States is 0.9997.

RESULTS

Since this child was positive for HIV-1 on two independent cultures of peripheral-blood lymphocytes obtained 32 days apart, as well as on a plasma culture (performed in a separate laboratory), it is highly unlikely that the early positive cultures from the infant resulted from inadvertent contamination with HIV-1. Nevertheless, to provide further confirmation, we analyzed nucleic acid sequences of PCR-generated clones of the HIV-1 DNA in the two cultures to determine whether the viruses were similar. Similarity would indicate that both came from the infant. We sequenced the V4 and C4 envelope regions of cultured virus (28 independent clones from samples obtained on day 19 and 5 from those obtained on day 51). As evidenced by visual examination of the viral sequences (Fig. 1), as well as by analysis with Clustal software10 (data not shown), the consensus sequences of the infant’s two isolates showed close homology, but there was a significant percentage of difference in comparison with other sequences in the database. In particular, the sequences differed substantially from the only laboratory strains commonly used in this laboratory (HIV-1JRCSF and HIV-1JRFL). This sequence analysis shows that the two viral isolates from the infant on day 19 and day 51 were very closely related. In addition, we compared the consensus sequence of the isolates from this infant with those of the first viral isolates from three other California-born infants in whom HIV infection was diagnosed during the same period (B1VC1, B3VC14, and B2VC3 in Fig. 1). The consensus sequence of the viruses from these three infants was substantially different from those of the two viral isolates from the infant.

To eliminate further the possibility of misclassifying the samples, we performed studies of genetic identity by co-amplifying six separate genetic markers by PCR amplification of peripheral-blood lymphocytes saved from the infant at 19 days of age (when the HIV-1 culture was positive) and at the age of 2½ years (when it was negative). The two samples contained identical six-locus genotypes: DQA1:4.1/4.1, LDLR:AB, GYPA:AB, HBGG:AB, D7S8:BB, and GC:AC. If these six loci are assumed to be statistically independent, the frequency of this genotype (obtained by multiplying the observed frequencies of the genotypes at each locus) was calculated to be 0.00013. If the expected frequencies of the genotypes are multiplied, the frequency of the six-locus genotype would be 0.0032, on the basis of Hardy–Weinberg assumptions. (This calculated difference in frequency is due to the difference between the observed and expected frequencies of DQA1:4.1/4.1 [0.055].)

Given one sample with this six-locus genotype, the probability of finding an identical genotype in a randomly selected white population would be less than 1 in 1000.

These data indicate that the two samples probably originated from the same person and that the likelihood of their originating from two different people is extremely low. Thus, it is highly unlikely that contamination in the laboratory or by another clinical sample could account for the two independent positive results in this child. In addition, the mother’s peripheral-blood mononuclear cells shared alleles at all six loci with those of the infant, in a manner consistent with this woman’s status as the biologic mother of this child.

We compared the nucleotide sequence of the virus obtained from the child with HIV-1 sequences from the mother. Unfortunately, no samples of the mother’s blood obtained at the time of delivery were available for analysis. Instead, samples of the mother’s lymphocytes 12 months after delivery were used in the sequence analysis. HIV-1 sequences isolated by PCR directly from the DNA of the mother’s peripheral-blood lymphocytes were analyzed as described in the case of HIV-1 DNA from the child. Since the mother’s peripheral-blood mononuclear cells were not cultured before analysis, there was wide heterogeneity of the HIV-1 sequence. Some of the maternal HIV-1 sequences were closely related to the principal sequence from the child’s samples (BC11-1 in Fig. 1). However, other HIV-1 sequences from the mother differed from those of the child. This result was expected, given the relatively extended time between the isolation of the HIV-1 DNA samples from the mother and from the child. Therefore, although it is difficult to assign with certainty an evolutionary relationship between the mother’s HIV-1 sequences and those of the infant, the similarities suggest such a relationship. When the results of the sequence analysis and the genetic typing are taken together, it is highly likely that both viruses found in the infant originated from the mother.

DISCUSSION

The data we report document the apparent clearance of HIV-1 from an infant infected perinatally. Questions remain about the mechanism involved in this clearance and about whether such an event may occur more frequently than we realize in infants born of HIV-infected mothers, although it may not be detectable because of a low viral load or insensitive assay methods. One potential explanation for transient HIV-positive cultures in an HIV-exposed infant may be the
persistence in the infant of infected maternal cells that are ultimately cleared. This is less plausible in this case because there were 19 days to the first positive culture from the infant and infection persisted in the infant for at least 1 month in association with plasma viremia, suggesting a primary infection. Even if there were infected maternal cells, they would not explain the failure of the virus to establish infection in the infant.

The infant had an HIV-1-negative blood sample at birth and a positive 19-day HIV-1 coculture, which suggests that he acquired the infection during birth, in ac-
cordance with current proposed working definitions of intrapartum and in utero transmission.11 The mother may have had primary or recent HIV infection during pregnancy for several reasons: her absolute CD4 cell counts were in the normal range, she was sexually exposed to a former intravenous drug user during gestation, and she remained asymptomatic with normal CD4 cell counts over the four subsequent years.

A number of studies of maternal–fetal HIV transmission describe other seroreverting infants with some unexplained positive results, including isolated transiently HIV-positive blood cultures, positive serum p24 antigen tests, or both. Borkowsky et al. described 4 of 14 seroreverting well children who had transient p24 antigen in their serum at the age of several months,12 and De Rossi et al. found 3 of 74 seroreverting infants with transiently positive results of PCR, viral culture, or both.13 In several large perinatal cohort studies, a small group (2.5 to 4.7 percent) of seronegative children born to HIV-positive mothers has been identified who had transiently positive viral markers.14,15 Baur et al. described one child with clearance of virus, but the follow-up of that infant was limited.15 A recent report found two seroreverting, clinically well children who had initially positive PCR results in early sequential samples, with subsequent tests that were repeatedly negative.16

Cases such as these have usually been dismissed as laboratory mistakes. In rare cases there have also been reports of infants who became seronegative at the age of 1 year and then became seropositive between 12 and 30 months of age.12 The child we describe, however, had no evidence of HIV infection by any laboratory or clinical measure at the age of 5 years. He has had no evidence of HIV infection by any laboratory or clinical measure at the age of several months,12 and De Rossi et al. found 3 of 74 seroreverting infants with transiently positive results of PCR, viral culture, or both.13 In several large perinatal cohort studies, a small group (2.5 to 4.7 percent) of seronegative children born to HIV-positive mothers has been identified who had transiently positive viral markers.14,15 Baur et al. described one child with clearance of virus, but the follow-up of that infant was limited.15 A recent report found two seroreverting, clinically well children who had initially positive PCR results in early sequential samples, with subsequent tests that were repeatedly negative.16

Our results indicate that clearance of HIV can occur and may be underrecognized. These findings may also have implications for the sexual transmission of HIV in adults. Previous studies of HIV-1–seronegative homosexual men who are at high risk have suggested that “abortive infection” may have occurred, although this issue has been very controversial.18–22 Recently, Clerici et al. detected T-cell responses to HIV peptides in homosexual men at high risk and in a subgroup of infants born of HIV-infected mothers, who all remained seronegative despite potential exposure to HIV. These studies suggested that early cellular immune responses, as measured by the proliferation of interleukin-2 to HIV-specific peptide antigens, may be associated with the absence of infection.23–25 Other reports have detected HIV-1–specific cytotoxic T-cell responses or lymphoproliferation in response to HIV antigens in the peripheral-blood cells of seroreverting infants born of HIV-infected mothers.21,26,27 Whether there is an immune response to transient infection or an exposure to defective virus or HIV antigens without infection is unknown. The child we studied had no detectable proliferative response in his peripheral-blood cells to HIV-1 MN or IIIB envelope antigens, but further studies with autologous and maternal virus are in progress.

Understanding the mechanism of clearance based on either the biologic characteristics of the virus or the immune response of the host could have profound implications for HIV-1 therapy and vaccine development. Potential exposure to low-dose virus or viral antigens in the presence of maternal antibody may only serve to immunize the infant and create a long-term specific immunity without the production of antibody, as has recently been proposed.28 Zack et al. have reported in vitro results indicating that in quiescent peripheral-blood mononuclear cells, the HIV-1 genome is represented in the form of partially completed reverse transcripts that are labile and have an intracellular half-life of approximately 24 hours before they are cleared without evidence of integration.29 Although there are no in vivo data, these data provide some laboratory rationale for the ability of the host to eliminate the virus in peripheral-blood lymphocytes with low-level exposure without immune stimulation.30 However, the child we studied had virus that was detectable for at least 32 days.

Further careful study of infants with potential exposure to HIV and evidence of clearance of the virus should provide important insights into the pathogenesis of HIV and explain further why over 70 to 80 percent of infants born to HIV-1–infected mothers escape infection.

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**References**


