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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 neuropsychological 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-

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sults of laboratory studies of the infant, including HIV cocultures; measurements of plasma viremia, p24 antigen (Abbott, Abbott Park, Ill.), and acid-dissociated, 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-dissociated, immune-complex-dissociated p24 antigen were negative in the infant, as were four HIV-1 IgA dot blot assays during the same period.⁴

Since the child 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.³ 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.⁶ 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 μ M TRIS and 1 μ M EDTA; pH, 8). Quantitative PCR was performed to determine the concentration of HIV-1 in the samples. With labeled primers M667 and M661, 1 μ g of DNA was subjected to 25 cycles of PCR.⁷ 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 5' primer, env22 (GTGGAGGGGAATTCCTTCTACTGTAA), and the 3' primer, env21 (GGTACCACCATCTCTTGTAAATAG),⁶ 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 *Eco*RI and ligated to the M13mp18 vector digested with *Eco*RI and *Sma*I. The ligated DNA was introduced into XL1-Blue cells (Stratagene, La Jolla, Calif.). Positive plaques selected by hybridization screening⁸ were sequenced with DNA sequencing kits (United States Biochemical, Cleveland). Sequences from the baby, the mother, and the HIV Database⁹ were assessed with the Clustal program.¹⁰ The nucleic acid sequences isolated from the mother and the infant have been deposited in the GenBank data base (accession numbers L33045 through L33074).

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 (GTCAGC-ACAGTACAATGTAC; positions 6953 to 6972 of HIV_{JRCSF}) and 3'RedE2 (GCACCCTCTTCTCTTTGCC; positions 7711 to 7730 of HIV_{JRCSF}) with inner primers 5'RedE3 (AATTCTAGATAGG-CCAGTAGTATCAACTC; positions 6982 to 7001 of HIV_{JRCSF}) and 3'RedE4 (AATTAAGCTTCACTTCTCCAATTGTCCCTC; positions 7636 to 7657 of HIV_{JRCSF}) and digestion with *Xba*II and *Hind*III. 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.*

TEST	PRENATAL	CORD BLOOD	DAYS AFTER BIRTH			MONTHS AFTER BIRTH						
			19	33	51	3	6	10	12-13	14	28	36-60
Infant												
HIV-1 culture PBL	—	Negative	Positive (10 ⁶ PBL)	Negative (10 ⁶ PBL)	Positive (10 ⁶ PBL)	—	—	—	Negative	Negative	Negative	Negative†
Plasma HIV-1 culture	—	—	—	Negative	Positive (100 TCID ₅₀)	Negative	—	—	—	—	—	—
p24 Antigen	—	—	Negative	Negative	—	Negative	—	—	Negative	Negative	—	—
ICD p24 antigen	—	—	Negative	Negative	Negative	Negative	—	—	—	—	—	—
PCR	—	—	—	Positive	—	—	—	—	—	Negative	Negative	Negative†
ELISA or Western blot assay	—	—	Positive	Positive	Positive	—	—	Losing bands	Negative	Negative	Negative	Negative
HIV IgA dot blot assay	—	—	—	Negative	Negative	Negative	Negative	—	—	—	—	—
Mother												
HIV ELISA	Positive	—	—	—	—	—	—	—	Positive	—	—	—
HIV-1 culture	—	—	Negative	—	—	—	—	—	Negative	—	Positive	—
PCR	—	—	—	—	—	—	—	—	Positive	—	Positive	—

*Dashes indicate not determined. PBL denotes peripheral-blood lymphocytes, TCID₅₀ median tissue-culture infective doses, ICD immune-complex-dissociated, and ELISA enzyme-linked immunosorbent assay.

†This test was repeated numerous times during this period.

Foster City, Calif.). In addition, known sequences from 27 North American HIV isolates, the BRU and OYI isolates from western Europe, and the JY1 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 AmpliType 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), glycoporphin 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 diversity in comparison with other sequences in the data base. In particular, the sequences differed substantially from the only laboratory strains commonly used in this laboratory (HIV-1_{JRCSF} and HIV-1_{JRFL}). 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

364	<p>Consensus C n s t q L F n s t W ? ? ? ? ? N ? . ? ? ? T ? ? ? ? ? n ? t ? ? n ? t ? I t L p C r I K q i i N m W Q e V g k A m Y a p P I ?</p> <p>BC11-1 CNSTQLFNSTW N G T E . . . K V N S T G . N I T L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>BC11-2 CNSTQLFNSTW N G T E . . . K V N S T G . N I T L P C R I K Q I I N M W Q E V G K A T Y A P P I R</p> <p>BC11-6 CNSTQLFNSTW N G T E . . . K V N S T G . N I T L P C R I K Q I I N M W Q E V G K A M Y A P # I R</p> <p>BC11-7 CNSTQ Q F N S T W N G T E . . . K V N S T G . N I T L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>BC11-9 CNSTQLFNSTW N G A E . . . K V N S T G . N I T L P C R I E Q I I N M W Q E V G K A M Y A P P I R</p> <p>BC11-14 CNSTQLFNSTW N G T E . . . K V N S T G . N I T L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>BC21-2 CNSTQLFNSTW N G T K . . . K V N S T G . N I \$ L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>MC10-4 CNSTQLFNSTW N G T E G S P . N . T E . N I T L P C R L K Q I I N L W Q E V G K A M Y A P P I K</p> <p>MC10-2 CNSTQLFNSTW N S T W N S T E G L P D N I T L P C R L K Q I I N M W Q E V G K A M Y A P P I K</p> <p>MC10-3 CNSTQLFNSTW N S T E G L P D N I T L P C R L K Q I I N M W Q E V G K A M Y A P P I K</p> <p>MC10-10 CNSTQLFNSTW N G T E G L P D N I T L P C R I K Q I I N M W Q E V G K A M Y A P P I K</p> <p>MC10-11 CNSTQLFNSTW N S T W N G T G G S P . N . T E . N I T F P C R I K Q I I N L W Q E V G K A M Y A P P I K</p> <p>B1VC1 CNSTKLFNSTWN A N S T W N D E T G L . N D T . I T L P C R I K Q I I N M W Q E V G K A M Y A P P I Q</p> <p>B3VC14 CDSTKLFNSTWN V . S N T E G L N N . . . T E G N G T . I I L P C R I K Q I V N M W Q E V G K A M Y A P P I R</p> <p>B2VC3 CNSTQLFNSTWN N S T E S L N D . . . T Q G N . . . S I T L P C I I K Q I I N M W Q E V G T A M Y A P P I G</p> <p>BAL1 CNSTPLFNSTW N V T E E . . . S N N T V E N N T . I T L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>HXB2 CNSTQLFNSTWF N S T W S . T E G S N N . . . T E G S D T . I T L P C R I K Q I I N M W Q E V G K A M Y A P P I S</p> <p>BRU CNSTQLFNSTWF N S T W S . T E G S N N . . . T E G S D T . I T L P C R I K Q F I N M W Q E V G K A M Y A P P I S</p> <p>NL43 CNSTQLFNSTWF N S T W S . T E G S N N . . . T E G S D T . I T L P C R I K Q F I N M W Q E V G K A M Y A P P I S</p> <p>SF2 CNSTQLFNSTWR L N H T . E G T K G N D T . I I L P C R I K Q I I N M W Q E V G K A M Y A P P I G</p> <p>SC CNSTQLFSSTW N G T E G S N N . . . T G G N D T . I T L P C R I K E I I N M W Q E V G K A M Y A P P I K</p> <p>MN CNSTPLFNSTWN G N N T W N N T T G S N . N I T L Q C K I K Q I I N M W Q E V G K A M Y A P P I E</p> <p>RF CNSTQLFNSTW N S T E G S N N T G G N D N . T . I T L P C R I K Q I V N M W Q E V G K A M Y A P P I S</p> <p>WMJ2 CNSTQLFNSTW N G T D I K G D N K N S T L I T L P C R I K Q I I N M W Q E V G K A M Y A P P I Q</p> <p>CDC4 CNSTQLFNSAWNV T . S N G T W S V T . . . R K Q K D T G D I T L P C R I K Q I I N R W Q V V G K A M Y A L P I K</p> <p>JH3 CNSTQLFNSTWL S N S T W N D T E G S N N T G G N D T I T L P C R I K Q I I N M W Q E V G K A M Y A P P I E</p> <p>BRVA CNSTQLFNSTWY R N T T G N I T E G N S P I T L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>ALA1 CNSTQLFNSTW N S T E G P K G S N N T E E N I T L P C R I K Q I V N M W Q E V G K A M Y A P P I E</p> <p>JRC5F CNSTQLFNSTW N D T E K S S G . . . T E G N D T . I I L P C R I K Q I I N M W Q E V G K A M Y A P P I K</p> <p>JRFL CNSTQLFNSTWN N N T E G S N N . . . T E G N . T . I T L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>SF162 CNSTQLFNSTW N N T I . . . G P N N T G T . I T L P C R I K Q I I N R W Q E V G K A M Y A P P I R</p> <p>SF33 CNSTKLFNSTWN E N S T W N A T G N D T . I T L P C R I K Q I I N M W Q E V G K A M Y A P P I E</p> <p>OYI CNSTQLFNSTW N D T T . . . R A N S T E V T . I T L P C R I K Q I V N M W Q E V G K A M Y A P P I S</p> <p>SBB CNSTKLFNSTWN D S S T W N V . E R S S K A E N D I L T L Q C R I K Q I V N M W Q E V G K A M Y A P P I R</p> <p>SBC CNSTQLFNSTWY S N N T W N . G T G G E N I T L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>TIBR1 CNSTELFNSTWN N G T F E G T N S T E R N D T . I T L Q C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>WM CNATQLFNSIWN G T N . . . G T E G S N N T G D S I T L P C R I K Q I I N M W Q E V G K A M Y A P P I A</p> <p>JY1 CNSTQLFNSTWN S S . T W N N D T L N S E G T . I K L P C R I K Q I I N M W Q E V G K A M Y A P P I E</p> <p>FLD21 CNSTQLFNSIWM T N G T W Y G T E G S N N T G E N I T L P C R I K Q I I N M W R E V G K A M Y A P P I R</p> <p>FLPA24 CNSTQLFNSIWM A N S T W K G S N N T G E N I I L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>FLPA25 CNSTQLFNSIWM A N S T W K G S N N T G E N I I L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>FLPA28 CNSTQLFNSIWI A N S T G K G S N N T G E N I I L P C K I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>FLPE21 CNSTQLFNSIWM A N G T T K G P N N T G E N I T L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p> <p>FLQ108 CNSTQLFNSTWK F E S T W N R T N G S E G N D T I T L P C R I K Q I V N M W Q E V G K A M Y A P P I R</p> <p>FLQ727 CNSTQLFNSTWN D T S T W N G T N E S N N T G G N D T L I L P C R I K Q I I N M W Q E V G K A M Y A P P I R</p>	406
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Figure 1. Sequence Homology of Independent HIV-1 Isolates from the Child.

DNA isolated from peripheral-blood mononuclear cells cocultured with peripheral-blood mononuclear cells from the child (BC) were amplified by PCR with the 5' primer, env22 (GTGGAGGGGAATTCTTCTACTGTAA), and the 3' primer, env21 (GGTACCACCATCTCT-TGTTAATAG).⁶ The nucleotide sequence was determined from independent M13 subclones of PCR-amplified regions of the HIV-1 V4/CD4-binding domain. The region sequenced corresponds to the region identified in the HIV data bases as extending from amino acid 364 to amino acid 406 of the env consensus sequence.⁹ Twenty-eight independent clones were sequenced from HIV-1 cultured from the blood sample obtained on day 19 from the infant, and five independent clones were sequenced from HIV-1 cultured from the sample obtained on day 51 (Table 1). Of the 28 clones from day 19, 23 were identical (represented by BC11-1; BC11-14 differs in the nucleotide sequence but is identical in the amino acid sequence). Of the five clones obtained from day 51, four were identical (BC21-2) and were identical to BC11-1, the majority type from day 19. Maternal HIV-1 sequences were derived similarly, except that PCR was performed directly on DNA from peripheral-blood mononuclear cells obtained 12 months after delivery. Two clones had sequences identical to MC10-2, and four had sequences identical to MC10-10. All the other maternal sequences were unique. B1VC1, B3VC14, and B2VC3 denote HIV-1 sequences from three infants born in California; BRU and OYI are isolates from western Europe; JY1 is from Zaire; and all other HIV-1 sequences are from North America.

The amino acid sequences for BC (the samples from the child, obtained on two days), MC (the mother), and selected HIV-1 sequences from the GenBank are shown in alignment, with the single-letter code for amino acids used. The consensus sequence for this region, as determined, is indicated at the top. In this sequence, uppercase letters indicate amino acids that are invariant, and lowercase letters indicate amino acids that are conserved in most HIV-1 strains. Question marks denote regions of nonconservation. The symbol "#" in BC11-6 denotes an insertion mutation with an extra C; CCC, the codon for P, is changed to CCCC. "\$L" is a deletion mutation in BC21-2; ACA, the codon for T, is changed to A because of the deletion of two nucleotides. Underscoring indicates a minor difference in the sequence. Therefore, the amino acids shown after these positions would, in reality, each undergo a frame shift.

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.¹ 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.^{3,14} 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 five years. He has had no lymphadenopathy for five years, and has had normal neurologic function and growth during that period. Of course, we cannot exclude the remaining possibility — that this child has silent or “hidden” HIV infection in some privileged site such as lymph nodes or the brain.¹⁷

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 un-

known. The child we studied had no detectable proliferative response in his peripheral-blood cells to HIV-1 MN or IIB 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.

We are indebted to Dr. Ray Apple and Henry Erlich of Roche Molecular Systems for kindly performing the assays of genetic markers; to S. Gillette, M. Dillon, and E.R. Stiehm for their help with this case; to T. Rossomando and W. Aft for assistance in the preparation of the manuscript; to N. Martin and D. Wara (of the University of California, San Francisco) for performing the HIV IgA assay, to Dr. Steve Wolinsky for his helpful comments and review of the manuscript; and to the late Elizabeth Glaser for her continued inspiration.

REFERENCES

- Daar ES, Moudgil T, Meyer RD, Ho DD. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N Engl J Med* 1991;324:961-4.
- Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N Engl J Med* 1989;321:1621-5.
- European Collaborative Study. Risk factors for mother-to-child transmission of HIV-1. *Lancet* 1992;339:1007-12.
- Martin NL, Levy JA, Legg H, Weintrub PS, Cowan MJ, Wara DW. Detection of infection with human immunodeficiency virus (HIV) type 1 in infants by an anti-HIV immunoglobulin A assay using recombinant proteins. *J Pediatr* 1991;118:354-8.
- Pang S, Koyanagi Y, Miles S, Wiley C, Vinters HV, Chen IS. High levels of unintegrated HIV-1 DNA in brain tissue of AIDS dementia patients. *Nature* 1990;343:85-9.
- Pang S, Vinters HV, Akashi T, O'Brien WA, Chen IS. HIV-1 env sequence variation in brain tissue of patients with AIDS-related neurologic disease. *J Acquir Immune Defic Syndr* 1991;4:1082-92.
- Lee H, Swanson P, Shorty VS, Zack JA, Rosenblatt JD, Chen IS. High rate of HTLV-II infection in seropositive i.v. drug abusers in New Orleans. *Science* 1989;244:471-5.
- Sambrook J, Fritsch E, Maniatis T. *Molecular cloning: a laboratory manual*. 2nd ed. Plainview, N.Y.: Cold Spring Harbor Laboratory Press, 1989.
- Myers G. *Human retroviruses and AIDS compendium*. Los Alamos, N.M.: Los Alamos National Laboratory, 1992.
- Higgins DG, Sharp PM. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. *Gene* 1988;73:237-44.

11. Bryson YJ, Luzuriaga K, Sullivan JL, Wara DW. Proposed definitions for in utero versus intrapartum transmission of HIV-1. *N Engl J Med* 1992;327:1246-7.
12. Borkowsky W, Krasinski K, Paul D, et al. Human immunodeficiency virus type 1 antigenemia in children. *J Pediatr* 1989;114:940-5.
13. De Rossi A, Ades AE, Mammano F, et al. Antigen detection, virus culture, polymerase chain reaction, and in vitro antibody production in the diagnosis of vertically transmitted HIV-1 infection. *AIDS* 1991;5:15-20.
14. Tovo PA, de Martino M, Gabiano C, et al. Prognostic factors and survival in children with perinatal HIV-1 infection: the Italian register for HIV infections in children. *Lancet* 1992;339:1249-53.
15. Baur A, Schwarz N, Ellinger S, et al. Continuous clearance of HIV in a vertically infected child. *Lancet* 1989;2:1045.
16. Pahwa S, Abrahms E, Tetali S, et al. Diagnosis of infection status in infants born to HIV infected women. In: Abstracts of the Ninth International Conference on AIDS/Fourth STD World Congress, Berlin, Germany, June 6-11, 1993. Vol. 1. London: Wellcome Foundation, 1993:306. abstract.
17. Pantaleo G, Graziosi C, Demarest JF, et al. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* 1993;362:355-8.
18. Imagawa DT, Lee MH, Wolinsky SM, et al. Human immunodeficiency virus type 1 infection in homosexual men who remain seronegative for prolonged periods. *N Engl J Med* 1989;320:1458-62.
19. Horsburgh CR Jr, Ou CY, Jason J, et al. Duration of human immunodeficiency virus infection before detection of antibody. *Lancet* 1989;2:637-40.
20. Pan LZ, Sheppard HW, Winkelstein W, Levy JA. Lack of detection of human immunodeficiency virus in persistently seronegative homosexual men with high or medium risks for infection. *J Infect Dis* 1991;164:962-4.
21. Borkowsky W, Krasinski K, Moore T, Papaevangelou V. Lymphocyte proliferative responses to HIV-1 envelope and core antigens by infected and uninfected adults and children. *AIDS Res Hum Retroviruses* 1990;6:673-8.
22. Soriano V, Hewlett I, Tor J, Clotet B, Epstein J, Foz M. Silent HIV infection in heterosexual partners of seropositive drug abusers in Spain. *Lancet* 1990;335:860.
23. Shearer GM, Sison AV, Rakusan T, et al. In vitro T cell immunity to HIV synthetic peptides and other antigens by peripheral blood leukocytes from HIV-infected women and by cord blood from their offspring. *J Cell Biochem Suppl* 1992;16E:112. abstract.
24. Clerici M, Berzofsky JA, Shearer GM, Tacket CO. Exposure to human immunodeficiency virus (HIV) type 1 indicated by HIV-specific T helper cell responses before detection of infection by polymerase chain reaction and serum antibodies. *J Infect Dis* 1991;164:178-82. [Erratum, *J Infect Dis* 1991;164:832.]
25. Clerici M, Giorgi JV, Chou CC, et al. Cell-mediated immune response to human immunodeficiency virus (HIV) type 1 in seronegative homosexual men with recent sexual exposure to HIV-1. *J Infect Dis* 1992;165:1012-9.
26. Cheynier R, Langlade-Demoyen P, Marescot MR, et al. Cytotoxic T lymphocyte responses in the peripheral blood of children born to human immunodeficiency virus-1-infected mothers. *Eur J Immunol* 1992;22:2211-7.
27. Pollack H, Zhan MX, Ilmet-Moore T, Ajuang-Simbiri K, Krasinski K, Borkowsky W. Ontogeny of anti-human immunodeficiency virus (HIV) antibody production in HIV-1-infected infants. *Proc Natl Acad Sci U S A* 1993;90:2340-4.
28. Salk J, Bretscher PA, Salk PL, Clerici M, Shearer GM. A strategy for prophylactic vaccination against HIV. *Science* 1993;260:1270-2.
29. Zack JA, Haislip AM, Krogstad P, Chen IS. Incompletely reverse-transcribed human immunodeficiency virus type 1 genomes in quiescent cells can function as intermediates in the retroviral life cycle. *J Virol* 1992;66:1717-25.
30. Bukrinsky MI, Stanwick TL, Dempsey MP, Stevenson M. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science* 1991;254:423-7.

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CORRECTION

Clearance of HIV in an Infant

To the Editor: Bryson and colleagues report the spontaneous clearance of human immunodeficiency virus (HIV) infection in a baby (March 30 issue).¹ Unfortunately, spontaneous clearance seems to be rare, and we have not observed it in any of 180 babies born to seropositive mothers.² We tested for the presence of HIV in these infants two to four times during the first two months of life by means of polymerase-chain-reaction (PCR) testing of peripheral-blood mononuclear cells and HIV culture. All 33 infants with positive tests at birth or at two to four weeks of life also had positive tests on subsequent occasions and were confirmed as having HIV infection.

To demonstrate the spontaneous clearance of HIV infection, two points must be established: a confirmed diagnosis of HIV infection early in life, and the absence of infection in the child at the present time. In the case reported by Bryson et al., the latter point seems more likely, given the five years of follow-up. However, the use of PCR to test for HIV-1 RNA in plasma may help detect low-level viremia produced by cells that are inaccessible by blood sampling.

The diagnosis of infection in this infant was based on two positive HIV cultures on days 19 and 51; a third culture, on day 33, was negative. In this very unusual clinical situation, direct PCR studies of uncultured peripheral-blood mononuclear cells could have confirmed the diagnosis and allowed further sequencing and accurate nucleic-acid comparisons,³ averting possible artifacts due to culture. Since cryopreserved peripheral-blood mononuclear cells were available for extensive genetic studies on day 19, they could be used to test for HIV-1 proviral DNA by PCR. A negative result would diminish the message of hope conveyed by this unique observation.

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References

1. Bryson YJ, Pang S, Wei LS, Dickover R, Diagne A, Chen ISY. Clearance of HIV infection in a perinatally infected infant. *N Engl J Med* 1995;332:833-838.
2. Krivine A, Firtion G, Cao L, Francoual C, Henrion R, Lebon P. HIV replication during the first weeks of life. *Lancet* 1992;339:1187-1189.
3. Wolinsky SM, Wike CM, Korber BT, et al. Selective transmission of human immunodeficiency virus type-1 variants from mothers to infants. *Science* 1992;255:1134-1137.

To the Editor: The report by Bryson et al. is a fascinating study of the possibility that HIV was eliminated from a host. However, we believe that another explanation must be considered.

It has been well documented that maternal-to-fetal engraftment of T cells occurs regularly in infants born with severe combined immunodeficiency.¹ This phenomenon may be a factor in the transmission of HIV. That T cells transferred from mother to infant are viable is shown by their ability to produce graft-versus-host disease.¹ Furthermore, in babies with severe combined immunodeficiency, maternal cells have been shown to be tolerated for up to 12 months after birth.¹

It is therefore possible that the HIV detected on days 19 and 51 in the infant described by Bryson et al. was that found in maternal cells. Only small numbers of maternal cells may be transferred to the fetus, resulting in the negative results of early PCR testing. Subsequently, such cells would proliferate in response to the infant's lymphocytes, resulting in increased expression of HIV. In an immunocompetent host, the maternal cells would be cleared. The result would be the apparent clearance of HIV infection in the host. The presence of HIV in maternal cells could also explain why a detectable proliferative response to HIV-1 was not found.

Genetic studies were performed to ensure that no error was made in sample labeling. Were these tests sufficiently sensitive to exclude the possibility that a low level of maternal DNA was present? Since the child was male, determination of the karyotype of the HIV-expressing cells could provide the answer.

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References

1. Pollack MS, Kirkpatrick D, Kapoor N, Dupont B, O'Reilly RJ. Identification by HLA typing of intrauterine-derived maternal T cells in four patients with severe combined immunodeficiency. *N Engl J Med* 1982;307:662-666.

The authors reply:

To the Editor: We based the diagnosis of HIV-1 infection in this infant on the presence of cultured virus in samples of peripheral blood obtained from the infant on two separate occasions — an approach that is consistent with the guidelines established by the National Institutes of Health AIDS Clinical Trials Working Group and the Centers for Disease Control and Prevention.^{1,2,3} DNA PCR assays with peripheral-blood mononuclear cells have entailed more problems with potential

laboratory contamination in other studies, although we have found the assay to be sensitive and specific.⁴ The infant had a positive result on DNA PCR testing, with a very low number of copies (3 per microgram of DNA), on one occasion and positive cultures on two occasions; only 1 in 1 million cells was infected. This result indicates that the infant had a very low viral load, as compared with other infected infants we and other investigators have followed sequentially during the first few months of life.^{4,5} We would have liked to perform all possible tests on this child, particularly on the early occasions; we were limited, however, by the small specimens available for retrospective testing and the need to gain the maximal amount of information.

We agree with Dr. Gompels and his colleagues that it is possible that infected maternal cells persisted in the infant's circulation. If the maternal cells were proliferating in the infant for several months, as the authors suggest, it would still not explain why the infant's own cells were not infected. The mother's very low viral load as evidenced by our inability to culture virus from her blood for over a year, and the presence of cell-free virus in the plasma of the infant at the time of the second positive culture (suggesting replication) make this possibility less likely.

We would also like to correct a statement in our article. Although we state that "some of the maternal HIV-1 sequences were closely related to the principal sequence from the child's samples" (page 835, righthand column, lines 35 to 37), as we state elsewhere in the article, it is not possible at this time to establish a relation between the mother's virus and that of the infant. This fact has no bearing on the central conclusion of our report; the infant was infected, as evidenced by the isolation of identical virus on two different occasions, and then became free of virus. The possibility that the virus was maternally derived has not been formally confirmed and is currently being addressed.

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References

1. Revised classification system in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep* 1994;43(RR-12).
2. Virology Technical Advisory Committee. *Virology manual for HIV laboratories*. Bethesda, Md.: AIDS Clinical Trials Group, September 1994.
3. McIntosh K, Pitt J, Brambilla D, et al. Blood culture in the first 6 months of life for the diagnosis of vertically transmitted human immunodeficiency virus infection. *J Infect Dis* 1994;170:996-1000.
4. Dickover RE, Dillon M, Gillette SG, et al. Rapid increases in load of human immunodeficiency virus correlate with early disease progression and loss of CD4 cells in vertically infected infants. *J Infect Dis* 1994;170:1279-1284.
5. Luzuriaga K, McQuilken P, Alimenti A, Somasundaran M, Hesselton R, Sullivan JL. Early viremia and immune responses in vertical human immunodeficiency virus type 1 infection. *J Infect Dis* 1993;167:1008-1013.