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Polymerase Chain Reaction for Studies of Mother to Child Transmission of HIV1 in Africa

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The feasibility and implications of the use of the polymerase chain reaction (PCR) assay in studies of HIV1 mother to child transmission in Africa were investigated. Uncultured leukocyte blood cells (PBL) obtained in Brazzaville (Congo) from newborns and infants (mean age = 27 weeks) of infected mothers were tested. HIV1 DNA sequences were identified in the PBL of six of eight newborns and 14 of 23 babies born to HIV1-positive mothers. In addition two of four babies, who at birth had been seropositive and subsequently were seronegative, were HIV1 DNA positive by PCR. This study demonstrates directly, therefore, a high rate of HIV1 transmission in Africa; it also indicates that PCR should be used for such epidemiological studies.

KEY WORDS: AIDS in Africa, perinatal transmission

INTRODUCTION

Human immunodeficiency virus (HIV) infection has now clearly emerged as a major health problem in Africa [Mann et al., 1988]. Indeed, rates of 2–20% of infection among the sexually active age groups have been reported in urban centers of central Africa [Piot et al., 1988]. The spread of the viral infection appears to be due mainly to heterosexual transmission. In this context, infection of babies born to infected mothers might play a major role in the epidemiology of HIV infection. There is, however, no definitive data on this issue despite several ongoing studies [Andiman et al., 1988; Chirmule et al., 1988; Ryder et al., 1988]. This is because of the difficulty of initiating studies as well as to the absence of reliable markers for identification of HIV-infected newborns [Blanche et al., 1986; Gaetano et al., 1987; Goetz et al., 1988]. In addition, clinical studies based on infant mortality can provide only indirect estimates of the rate of transmission.

The polymerase chain reaction (PCR) has been shown recently to be a highly sensitive and direct tool for HIV1 DNA analysis in infants born from HIV-infected mothers [Hart et al., 1988; Lauré et al., 1988; Ou et al., 1988]. It is likely therefore that PCR may prove to be valuable for the evaluation of HIV infection in babies in Africa. We have, consequently, investigated the feasibility of this approach using samples obtained in Brazzaville (Congo) in the context of a prospective study of infant survival associated with HIV1 maternal infection. This approach was also used to analyze whether differences in epidemiological features of HIV infection in Europe and Africa might be associated with different rates of HIV1 DNA-positive babies.

PATIENTS AND METHODS

Patients

Two groups of subjects were studied (Table I) whose mothers were recruited either in maternity hospitals or mother-child clinics in Brazzaville. A 4% prevalence rate of anti-HIV1 was previously observed in this area [Lallemant et al., 1988]. Group I included eight newborns of mothers anti-HIV1 positive at the time of delivery. Blood samples were obtained 1 day after birth. Group II included 23 babies (11–48 weeks old, mean age = 27 weeks) born from HIV1-infected mothers. Nine of these had clinical manifestations (such as adenomegaly, hepatomegaly, weight loss, failure to thrive, pneumonia) consistent with HIV infection; four of them had clinical symptoms consistent with AIDS.

Methods

Serological tests. Blood samples from mothers and infants were tested for anti-HIV1 antibodies by enzyme

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TABLE I. HIV1 DNA Detection With the Polymerase Chain Reaction Assay in Peripheral Blood Leukocytes

Patients (Age)	Serology	HIV1 DNA			
		Overall result	Polymerase P3 P4	Polymerase P7 P8	Envelope P9 P10
Group I: Newborns (8)					
1 (1 day)	+	+	+	+	+
2 (1 day)	+	−	−	−	−
3 (1 day)	±	−	−	−	−
4 (1 day)	+	+	+	−	+
5 (1 day)	+	+	+	+	+
6 (1 day)	±	+	−	−	+
7 (1 day)	+	+	−	−	+
8 (1 day)	+	+	−		+
Group II: Babies (23) Weeks					
1 (24)[b]	±[c]	+	+	−	−
2 (16)[a]	+	±	+	+	±
3 (11)[b]	+	+	±		+
4 (28)[a]	ND	±	+	−	+
5 (14)[a]	+	+	+	−	±
6 (24)[a]	±[c]	±	+	+	+
7 (22)[b]	±	±	+	−	−
8 (28)[a]	+	+	+	+	+
9 (26)[b]	−	±	−	−	+
10 (41)[b]	−	−	−	−	−
11 (24)[b]	±[c]	−	−	−	−
12 (27)[b]	±[c]	−		−	
13 (14)[b]	+	−	−	−	−
14 (32)[a]	±[c]	+	−	±	+
15 (40)[b]	ND	−	−		−
16 (24)[b]	ND	+	±	−	+
17 (36)[b]	ND	−	−	−	−
18 (20)[a]	ND	−	−	−	−
19 (32)[a]	±	+	+	+	+
20 (48)[b]	−	−	−	−	−
21 (20)[a]	+	+	+	−	±
22 (32)[b]	ND	−	−	−	−
23 (48)[b]	−	+	+	+	+

[a]Indicates the presence of clinical symptoms possibly related to HIV infection (babies 6, 8, 19, and 21 had clinical features consistent with AIDS).
[b]Indicates absence of symptoms.
[c]± Indicates presence of only one HIV1 protein revealed on Western blot analysis.

immunoassay (ELAVIA, Diagnostic Pasteur) and Western blot analysis (Dupont de Nemours). Results were considered positive if the serum contained antibodies against at least two envelope glycoproteins.

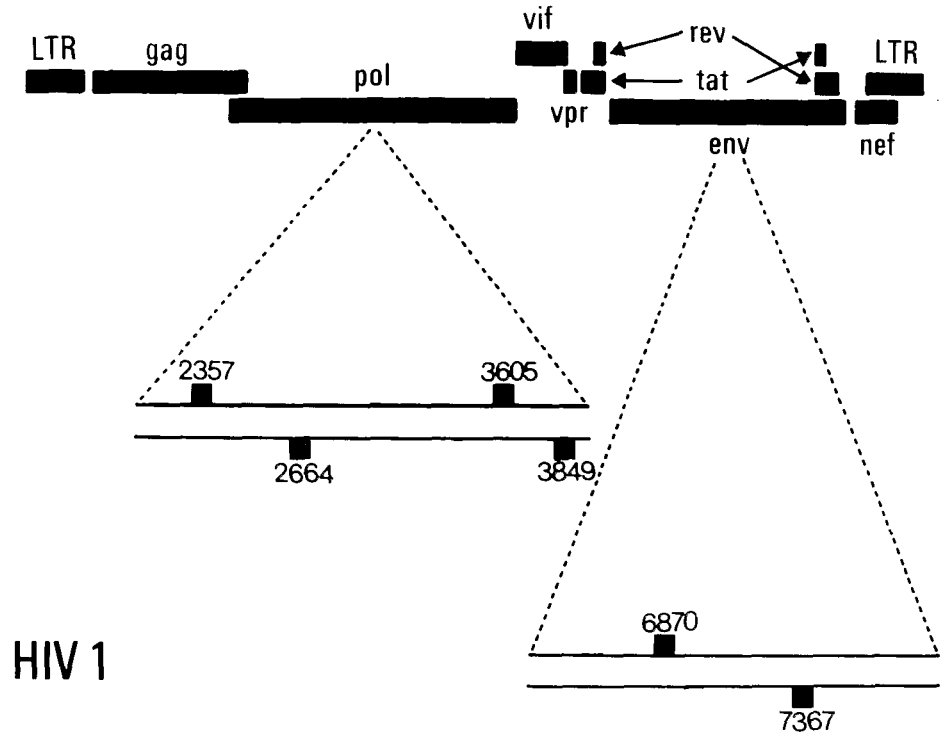
Peripheral blood leukocyte (PBL) preparation. Because of the low amount of blood available (1–2 ml), in particular from newborns, total leukocyte preparations were used. The cells were isolated after lysis of the red blood cells with ammonium chloride (9 g/l) for 1 hour at 8°C with constant agitation, followed by centrifugation (2,000*g*).

Cellular DNA purification. Leukocytes were resuspended in 2 ml of lysis buffer (Tris-HCl, pH 8, 10 mM; NaCl 10 mM; SDS 0.5%; EDTA, pH 8, 10 mM) and treated with proteinase K (300 μg/ml) at 37°C overnight. DNA was then extracted with phenol/chloroform and precipitated with ethanol [Pasquinelli et al., 1986].

Polymerase chain reaction. PCR was performed as previously described [Laure et al., 1988] with 3 μg of cellular DNA in a final volume of 50 μl. After 40 cycles of amplification, a Southern blot analysis was performed using oligonucleotide probes [Laure et al., 1988; Thiers et al., 1988]. A combination of three different sets of primers located in either the polymerase or the envelope gene were used (Fig. 1).

Because of the technical problems in obtaining blood in good condition from these newborns and babies, many of the samples were coagulated. To test for poor cellular DNA preparations, each DNA sample was assayed both with the HIV1 primers and with primers specific for the β-globin locus (primers PC03–PC04 [Saiki et al., 1985][Fig. 2]). Samples that scored negative with the β-globin primers after migration and ethidium bromide staining in agarose gel were further purified by repeating phenol-chloroform and ethanol preparative steps.

The specificity of the results was shown by their reproducibility in duplicates and the negative controls used in each assay. Moreover, highly stringent conditions were used for the hybridization procedures. Negative controls included tests on cellular DNA from normal blood donors as well as a PCR reaction performed



PRIMER OR PROBE	SEQUENCE (5′–3′)	LOCALIZATION IN HIV1*
P3 PRIMER	TGGGAAGTTCAATTAGGAATACCAC	Pol 2357–2381
P4 PRIMER	CCTACATACAAATCATCCATGTATTG	Pol 2639–2664
PROBE P3/P4	ATGAGACACCAGGGATTAGATATCAGTACAATGTGCT	Pol 2505–2541
P7 PRIMER	GGAATCATTCAAGCACAACC	Pol 3605–3624
P8 PRIMER	TCACTAGCCATTGCTCTCC	Pol 3831–3849
PROBE P7/P8	GCACACAAAGGAATTGGAGGAAATGAACAAGTAGAT	Pol 3707–3742
P9 PRIMER	ATCCTCAGGAGGGGACCCAGAAATT	Env 6870–6894
P10 PRIMER	GTGCTTCCTGCTGCTCCCAAGAACCC	Env 7342–7367
PROBE P9/10	TCAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGT	Env 7120–7158

*Isolate LAV Wain-Hobson et al., 1985

Fig. 1. Localization on HIV1 genome and nucleotide sequence of primers and probes used for PCR.

without addition of cellular DNA to identify any contamination of the batch of primers. In addition, we were also able to demonstrate that the primers used in this study did not identify HIV2 isolates (not shown). When strictly fulfilling all these criteria, positive results obtained with one set of primers were considered as identifying HIV1 DNA sequences.

RESULTS

For the 31 samples included in the study, amplification using β-globin primers showed clearly positive results after ethidium bromide staining (Fig. 2). However, repeated DNA purification was initially necessary to obtain interpretable results. By using HIV1 specific primers, located in the polymerase and envelope viral genes, positive results could be demonstrated after hybridization of the Southern blots with oligonucleotide probes (Fig. 2); the specificity of the results was assessed by repeated testing and by the absence of signals for the negative controls.

Among the eight newborns in group I, born to HIV1-infected mothers, six had detectable HIV1 DNA sequences in the PBL. In group II, 17 of 23 sera were available at the time of PCR assay (Table I). HIV1 DNA was detected in PBL of 14 of 23 babies. Among the 14 babies positive by PCR, ten had positive or incomplete and two had negative Western blots. Among the nine babies negative by PCR, three (24, 27, and 14 weeks old) had positive or incomplete and two (41 and 48 weeks old) had negative Western blots (Table I). Therefore, two of four babies (26 and 48 weeks old), who were seronegative at the time of PCR test, had HIV1 DNA in PBL, these two individuals being asymptomatic. Eight (including the four with symptoms con-

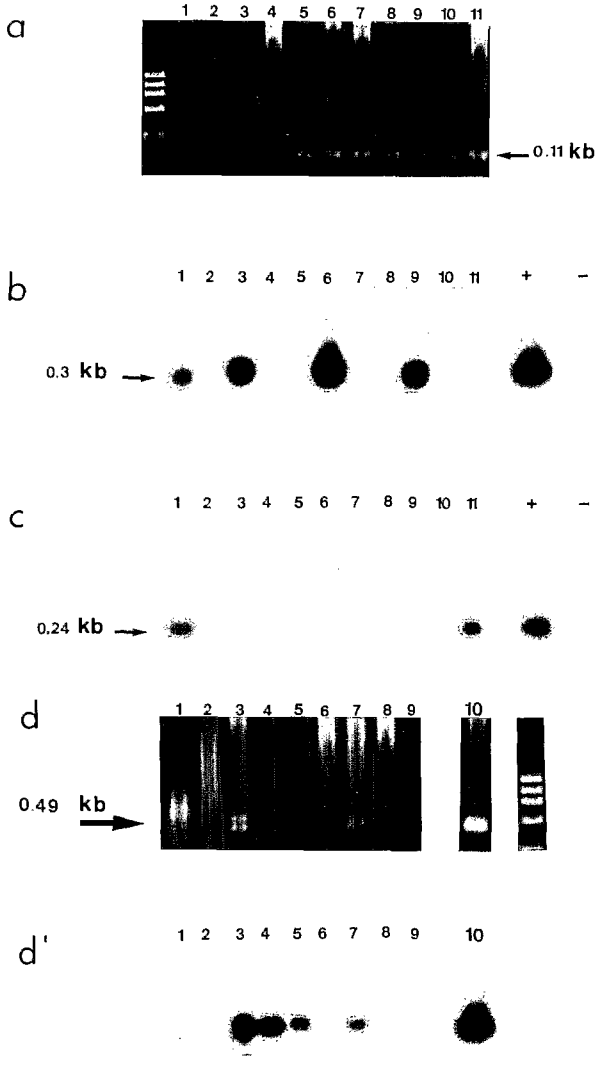


Fig. 2. Electrophoretic and Southern blot analysis of amplified DNA sequences. **a:** Electrophoretic analysis on 1.5% agarose gel of amplified DNA with β-globin primers PC03–PC04. **b,c:** Autoradiography after Southern blot transfer and hybridization with oligonucleotide probes. PCR with P3 P4 primers (b) and P7 P8 primers (c). **Lanes 1,3,6,9** (b): Samples from newborns and babies positive for P3 P4 primers. **Lanes 1,5,11** (c): Samples from newborns and babies positive for P7 P8 primers. **Lane 2** (a): Cellular DNA without detectable amplification with β-globin primers. + and −, positive and negative controls. **d,d':** Electrophoretic analysis on 1.5% agarose gel (d) and autoradiography (d') of amplified DNA with P9 P10 primers. **Lanes 3,4,5,7** (d,d'): Samples from newborns and babies positive for P9 P10 primers. **Lanes 8,9** (d,d'): Negative controls (8 = normal cellular DNA and 9 = amplification without cellular DNA). **Lane 10** (d,d'): Positive control.

sistent with AIDS, see Table I) of the 14 HIV1 DNA-positive and one of nine HIV DNA-negative babies had clinical features likely to be related to HIV infection.

The results obtained with the three sets of primers markedly differed (Table I); indeed seven of 31 samples yielded positive results with all three sets of primers as compared to seven of 31 which were positive with two sets of primers but negative with the third, and the remaining six of 31 samples were only positive with one set.

DISCUSSION

This study provides direct information on mother to child transmission of HIV1 in Africa and suggests that a very high rate should be expected. In addition, it shows that the polymerase chain reaction assay can be successfully used for such studies performed in Africa, despite technical problems inherent to these kinds of investigations. Indeed, the tests were performed using total blood leukocyte preparations collected and prepared in difficult circumstances. In such conditions, inhibitors of Taq polymerase might copurify with the genomic DNA [de Franchis et al., 1988]; in particular, hemoglobin has been shown to inhibit in vitro amplification (J. Sninsky, Cetus Company, personal communication). Therefore, it was important to assess critically the methodology before employing the PCR assay for such studies. Because of potential technical problems, the PCR procedure can currently be performed only in research laboratories. Repeated DNA purification resulted in DNA of sufficient quality as shown by in vitro amplification using β-globin primers. In addition, three sets of HIV1 specific primers were used since previous studies [Laure et al., 1988] have indicated that testing with only one set may lead to false-negative results.

Among 31 babies born to HIV1-infected mothers, 20 (64%) were found to contain HIV1 DNA sequences in blood leukocytes. They included six of eight newborns and 14 of 23 babies 11–48 weeks old. Thus, in this investigation, we directly demonstrate a high rate of mother to child HIV1 transmission. This could not be predicted by the serological status of the babies because of the presence of mothers anti-HIV1 [Blanche et al., 1989]. The figure is higher than that previously observed in Europe: indeed, clinical studies as well as PCR assays performed in France and Switzerland suggested a 30–40% rate of transmission [Laure et al., 1988; Blanche et al., 1989; B. Mach, personal communication]. A further point will be to assess this observation on a large number of samples with standardized technical conditions among different laboratories. Additional investigations, possibly including HIV1 mRNA detection, will be needed to determine whether the high rate of HIV1 transmission in Africa might be linked to an enhanced viral replication in the infected mothers. It will also be important to analyze the clinical implications of positive results obtained with only one set of primers. Indeed, the rates of positivity were 45% when including positive results with two or three pairs of primers as compared to the 64% overall positivity. Studies are also required to examine whether infection occurs at birth and/or during the first months of life (in particular by breast feeding).

The present work also led to the identification of HIV1 DNA sequences in PBL from asymptomatic babies (26 and 48 weeks old) who were anti-HIV1 nega-

tive at the time of PCR analysis. This finding raises the issue of latent HIV1 infections, undetectable by conventional serological tests—a fact that might have important clinical and epidemiological implications [Lauré et al., 1988].

In conclusion, the PCR technique, performed under strictly controlled conditions, promises to be of great importance for HIV studies in Africa. Namely, 1) it is able to give a direct estimate of the HIV1 mother to child transmission rate, and 2) it clearly demonstrates HIV1 infection without the difficulties inherent in the interpretation of serological assays [Josse et al., 1988].

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