Concordance of Polymerase Chain Reaction with Human Immunodeficiency Virus Antibody Detection

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To evaluate the correlation of detection of human immunodeficiency virus (HIV) by polymerase chain reaction (PCR) with detection of HIV antibody, 271 simultaneous serum and peripheral blood mononuclear cell samples were examined from 242 persons whose activities placed them at increased risk for HIV infection: 142 from homosexual men, 86 from hemophilic men, and 43 from heterosexual partners of HIV-infected persons. PCR was performed using the *gag* region primer pair SK38/39 and the *env* region primer pairs SK68/69 and CO71/72. Amplified HIV DNA was detected using specific oligomer probes. Of 63 HIV antibody–positive samples, 58 (92%) had HIV DNA by PCR. Of 208 HIV antibody–negative samples, 7 (3.4%) had HIV DNA by PCR. On follow-up, 4 of of the latter persons were seropositive when next tested; 2 were well and antibody- and PCR-negative; 1 had died of a stroke before retesting. Thus, PCR detects HIV in most antibody-positive persons; detection is increased by use of multiple primer pairs. PCR-positive antibody-negative specimens may indicate HIV infection in which antibody has not yet developed or may be false-positive PCR results. When PCR is discordant with HIV antibody, testing of additional specimens and clinical follow-up are necessary to assess HIV infection status.

Since the introduction of the enzyme immunoassay (EIA) with Western blot confirmation for detection of antibody to the human immunodeficiency virus (HIV) in 1985, this method has been the standard for identification of HIV-infected individuals. However, some persons may be HIV-infected without antibody, either because it is early in the course of infection [1] or because no HIV antibody was detected for a prolonged period after HIV infection [2, 3]. Such cases of HIV infection can be identified by detection of HIV by virus culture or by antigen detection, but HIV culture is expensive and time-consuming and antigen detection has low sensitivity, particularly in the asymptomatic person [4].

Although HIV DNA is present in peripheral blood mononuclear cells of infected individuals, it often is present in such small amounts that direct detection with DNA probes is not feasible. We recently described the use of the polymerase chain reaction (PCR) technique for amplification of DNA from peripheral blood cells of HIV-infected persons so that detection can be performed with radioactively labeled probes [5].

In our earlier study, we found that PCR was quite sensitive in detecting HIV in persons with AIDS or severe AIDS-related symptoms, but few samples were tested from HIV-infected persons who had mild symptoms or were asymptomatic. Since such subjects may be less likely to be culture- or antigen-positive, we extended and refined our PCR procedure for HIV detection in 271 samples from HIV antibody–positive persons without AIDS or severe AIDS-related symptoms and from HIV antibody–negative persons whose activities placed them at increased risk for acquisition of HIV infection.

Materials and Methods

Patient samples. Cell and serum specimens were collected between 1984 and 1988 from participants in four prospective studies sponsored by the Centers for Disease Control (CDC): the Boston Partners Study, a prospective study of 329 homosexual and bisexual men and their sex partners [6]; the San Francisco City Clinic Cohort Study, a prospective study of homosexual and bisexual men who originally participated in studies of hepatitis B between 1978 and 1980 [7]; the Transfusion-Associated AIDS Study, a multicenter study of persons who became HIV-infected through blood transfusion or their sex partners [8]; and the Hemophilia/AIDS Collaborative Cohort Study, a prospective study of men with hemophilia and their wives [9]. Samples were tested to assess the frequency of HIV DNA in specific populations at risk and therefore are not representative of the overall study populations. Persons were excluded if they had AIDS or severe AIDS-related symptoms; these included persistent generalized lymphadenopathy (CDC class III), fever, weight loss, or diarrhea (CDC class IV-A), thrush, oral hairy leukoplakia, herpes zoster, *Salmonella* bacteremia, nocardiosis, or tuberculosis.

Laboratory procedures. Serum specimens were assessed with commercially available EIA (Abbott Laboratories, Chicago, or

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Genetic Systems, Seattle) and confirmed by immunofluorescence assay or Western blot interpreted by standard criteria [10]. Serum specimens originally tested with pre-1987 test kits were confirmed with currently available test kits.

Detection of HIV DNA by PCR. Peripheral blood mononuclear cells were separated by centrifugation on a ficoll-hypaque gradient and frozen in 10% dimethyl sulfoxide (DMSO) at −170°C until assays were performed. DNA was isolated from cells by lysis with a buffer containing 10 m*M* Tris-HCl, pH 8.3, 2.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.45% nonidet P-40, 0.45% Tween 20, 100 μg/ml gelatin, and 100 μg/ml proteinase K; the final concentration of the cells in the lysis solution was 6×10^6/ml.

Positive displacement pipettes were used to minimize sample carryover, and samples were prepared in a dedicated externally vented hood. The DNA preparation was incubated at 56°C for 60 min, followed by heat-inactivation of proteinase K at 95°C for 10 min. The DNA preparation was then subjected to PCR using the following procedure: A primer pair for the *gag* region of HIV-1, SK38/39 [5, 11], was used to subject 1 μg of the DNA preparation (equivalent to 1.5×10^5 cells) with 2.5 units of *Thermus aquaticus* DNA polymerase (provided by Cetus, Emeryville, CA) to 35 rounds of PCR in a volume of 100 μl. Reagent concentrations of the final reaction mixture were: 10 m*M* Tris-HCl, pH 8.3; 200 μ*M* (each) dATP, dCTP, dGTP, and thymidine triphosphate; 1 μ*M* (each) SK38 and SK39; 2.5 m*M* $MgCl_2$; 50 m*M* KCl; and 100 μg/ml gelatin. Cycling conditions were room temperature or 60°C (after the first cycle) to 95°C, 90 s; 95°C, 20 s; 95°C to 55°C, 90 s; 55°C, 30 s; 55°C to 60°C, 120 s.

Amplified HIV DNA was detected using the ^{32}P end-labeled *gag*-specific oligomer SK19 [5]. Samples were hybridized with the labeled probe in solution; these were then digested with *Bst*NI to produce a labeled cleavage product that was visualized by electrophoresis on a 20% polyacrylamide gel. Autoradiography, revealing the labeled diagnostic fragment, indicated a positive test result. A second primer pair, SK68/69 [5], for the *env* (gp41) region of HIV-1, was used if SK38/39 was negative. An end-labeled *env*-specific oligomer, SK70 [5], was used as a probe for detecting the amplified *env* region product. Samples were examined in duplicate; only if both PCR reaction products were positive was the specimen reported as positive. Positive and negative controls were included in each set of samples tested, and PCR laboratory personnel were unaware of the results of antibody testing of patient specimens.

In cases where antibody-positive samples were not PCR-positive with either SK38/39 or SK 68/69, samples were tested with a second *env* region primer pair, CO71/72, and probed with the probe CO75 [12]. This primer pair recognizes sites on the gp120 region of the envelope gene. Since hybridization of this probe with the amplified segment does not yield a restriction cleavage site, hybridization was performed on a nitrocellulose paper (slot-blot method) [12]. Positive and negative controls were included in each experiment; samples that hybridized to yield a stronger signal than the negative control were considered positive.

Results

The characteristics of the persons studied and their antibody status are shown in table 1. Twenty-seven homosexual men and 12 hemophilic men were selected for testing because they were known to have subsequently seroconverted [13]. Seventy-eight homosexual men were selected because they had probable exposure to HIV-infected persons (>250 lifetime sexual partners). They had been followed a mean of 25 ± 13 months since the date of the sample tested without developing HIV antibody or clinical evidence of HIV infection.

Table 1. Characteristics of persons studied.

Group (*n*)	HIV antibody status: Positive	Negative	Total
Homosexual men (117)	33	109	142*
Hemophilic men (82)	28	58	86*
Heterosexual partners of Transfusion-associated AIDS cases (18)	2	16	18
Hemophilic men (25)	0	25	25
Total (242)	63	208	271

* Some persons initially seronegative were later tested when seropositive.

Table 2. Concordance of human immunodeficiency virus (HIV) antibody and detection of HIV DNA by polymerase chain reaction (PCR).

Subjects	Antibody+ PCR+/total (%)	Antibody− PCR+/total (%)
Homosexual men	31/33 (94)	4*/109 (3.7)
Hemophilic men	25/28 (89)	3/58 (5.2)
Heterosexual partners	2/2 (100)	0/41 (0)
Total	58/63 (92)	7*/208 (3.4)

NOTE. + = positive − = negative.
* Includes two false-positive PCR results.

The replicate samples were discordant in only three cases; in each of these the replicate showing a labeled cleavage product was extremely faint. However, because no labeled product could be seen on the replicate gel, the sample was considered negative. All three of these samples were from HIV-seronegative persons.

Overall, HIV DNA was detected by PCR in 58 (92%) of 63 HIV antibody–positive samples (table 2). Of the 58, 51 were detected by SK38/39. No further positives were identified by SK68/69; however, seven additional positives were detected with the CO71/72 primer pair. Thus five persons had PCR-negative samples that were negative with all three primer pairs. PCR of human leukocyte antigen (HLA) gene sequences was positive in all five of these samples. A subsequent cell sample was available from one of these men and this was also PCR-negative. Subsequent serum samples from four of these men were also HIV antibody–positive; the fifth was lost to follow-up.

HIV DNA was detected in 7 (3.4%) of 208 samples in which there was no detectable HIV antibody. These samples were from 7 different persons. Two homosexual men and 2 hemophilic men were seropositive when tested 5, 11, 12, and 21 months later. A third hemophilic man had an intracerebral

hemorrhage 3 months after being tested and died before he could be reevaluated. He had been exposed to known HIV-contaminated lots of factor VIII and had 266 helper T cells/mm³.

The other two PCR-positive, seronegative persons were homosexual men whose cell samples were obtained in 1985. Both were also HIV p24 antigen–negative at that time. The extracted DNA from the first patient was positive with SK38/39 but not with SK68/69 or CO71/72. A repeat sample from the same date in 1985 was HIV-negative on PCR with all three primer pairs (PCR was positive for HLA). Follow-up cells in 1987 and 1988 were also HIV-negative on PCR with all three primer pairs but HLA-positive on PCR. He remains seronegative, antigen-negative, and asymptomatic, with normal numbers of T helper cells. The second man also had a repeat cell sample from 1985 tested; it was positive by PCR with all three primer pairs. Follow-up cells in 1988 were HIV-negative on PCR with all three primer pairs (HLA was positive on PCR). This man has also remained seronegative, antigen-negative, and asymptomatic with normal numbers of T helper cells.

Discussion

Identification of HIV infection by direct detection of HIV has several theoretical advantages over identification of infection by antibody detection. Antibody takes weeks to months to develop [1, 13] and may not develop in all infected persons [2, 3]. Also, assessment of HIV infection by virus culture or antigen detection, while quite specific, is expensive and time-consuming and may lack the necessary sensitivity to assure a patient that he or she is uninfected. We compared detection of HIV DNA by PCR with HIV antibody detection to assess the correlation of these two tests and to assess the utility of PCR in identifying HIV infection in persons who are HIV antibody–negative despite activities that put them at risk for infection with HIV.

PCR detected HIV in 58 (92%) of 63 HIV antibody–positive samples. Our previous report suggested that the use of multiple primer pairs would increase the sensitivity of PCR [5], and this was also true in the present study. Why then did PCR fail to detect HIV DNA in five seropositive persons? While improper storage of samples might lead to degradation of DNA and a false-negative result, this was excluded by successful PCR amplification of HLA gene product in the samples. A second possibility, that variation in HIV sequences targeted by the primer pairs had resulted in elimination of the enzyme restriction site, was investigated by performing direct gel analysis after solution hybridization; none of the samples were positive.

Other studies, using the same primer pairs, have reported the ability to detect HIV DNA by PCR in 97%–99% of HIV antibody–positive persons [2, 14]. However, the patients, where specified, had more advanced HIV disease than the patients in the present study. We believe the most likely explanation for failure to detect HIV DNA in a seropositive person is the presence of HIV DNA at a level below the sensitivity of the PCR amplification technique. Since low levels of HIV are more likely to be found in asymptomatic patients [15], concordance between PCR and antibody might be expected to be less in our study population.

In 208 samples from HIV-seronegative persons, PCR detected HIV DNA in 7. Four persons had seroconverted when seen at the next study visit. A fifth PCR-positive, seronegative sample was from a hemophilic man studied in 1984 who died 3 months later of a cerebral hemorrhage. It is possible that he was in the process of seroconverting, but this could not be determined. While we detected 4 (and possibly 5) seroconverters in 202 seronegative persons with behaviors that put them at increased risk of acquiring HIV infection, 39 (19%) of the 202 persons were specifically selected because they had seroconverted at the time of their next sample. Because this rate of seroconversion greatly exceeds that of any group yet reported, the apparent rate of PCR-positive antibody-negative infections, 4 (2%) of 202, cannot be taken as an estimate of the frequency of PCR-positive antibody-negative infections in even the highest risk population.

Two other seronegative persons had HIV DNA detected; both were homosexual men with >500 lifetime sexual partners and thus had presumably been exposed to HIV; however, both remain antibody-negative, PCR-negative, and well 3 years later. These two positive PCR results must therefore be considered false-positives.

False-positive PCR results may be caused by contamination with HIV DNA during processing or by specimen mislabeling. Contamination can occur if minute amounts of HIV DNA are aerosolized or carried over on pipette plungers. For this reason, we routinely use positive displacement pipettes and process samples in a dedicated externally vented hood. Despite these precautions, 2 (3%) of 65 PCR-positive results were false-positives. It is also possible that the positive PCR results before seroconversion were false-positives, since exposures to HIV continued to occur after the first sample was obtained. Thus neither PCR nor antibody testing is completely specific for detecting the presence of HIV infection. Whenever the two are discordant, testing of a second sample and clinical follow-up are advised.

False-positive test results might be reduced by a stricter definition of PCR positivity, such as a positive result with primer pairs from two or three gene regions. This would have eliminated one of our two false-positives; however, such a definition would greatly increase laboratory work load. Moreover, the number of indeterminate test results would increase, making clinical interpretation difficult.

The two men with false-positive PCR results were from a group of 78 seronegative homosexual men selected because of extensive high-risk exposure to HIV. They were the only men in this group to have a positive PCR test. Moreover, 41 antibody-negative heterosexual partners of HIV-infected per-

sons were also PCR-negative. Thus the persistence of HIV infection without the development of antibody, as has been reported by others [2, 3], was not seen in our study. We conclude that this phenomenon is uncommon, even in persons with many exposures to HIV.

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