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Prevalence of Human Immunodeficiency Virus Type 1 DNA in Hemophilic Men and Their Sex Partners

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Polymerase chain reaction (PCR) was used to detect human immunodeficiency virus (HIV)-1 DNA in peripheral blood mononuclear cells to assess in hemophilic men whether any were HIV-seropositive but uninfected or seronegative but infected and in seronegative sex partners of seropositive hemophilic men whether any were infected. Of 40 seropositive men, 38 (95%) were PCR-positive; one was PCR-indeterminate and one PCR-negative. None of 41 seronegative men who used only donor-screened, virus-inactivated coagulation factor products were PCR-positive. However, two of six who received noninactivated products were PCR-positive; one had low T-helper cell counts and died of unrelated causes and the other had seroconverted 11 mo later. PCR with a second primer pair also detected HIV-1 DNA in these two men. None of 25 seronegative female sex partners of seropositive men, including six men with AIDS and seven with AIDS-related symptoms, were PCR-positive. These data suggest that most seropositive hemophilic men are HIV-infected; whether some are infected with defective virus remains to be resolved as does the infection status of seropositive PCR-negative men. Identification of two seronegative PCR-positive men supports the possibility that HIV-1 DNA can be detected before seroconversion.

Hemophilic men receiving coagulation factor concentrates produced from US plasma donations between about 1980 and 1984 were at high and increasing risk of infection with human immunodeficiency virus (HIV) [1, 2], indicating widespread and increasing contamination of these products. Since 1985, donors of coagulation factor products have been screened for HIV antibodies and these products virus-inactivated; however, most hemophilic patients using coagulation factor products made from US plasma donations had already been infected with HIV, and an increasing proportion of those so infected are developing AIDS [3]. We used a new technique, polymerase chain reaction (PCR) [4, 5], to detect HIV-1 genetic material and address three recurring questions concerning the hemophilic population.

First, did any HIV-seropositive hemophilic persons seroconvert because of the presence of disrupted HIV rendered noninfectious by processing rather than infection with viable virus [1, 2, 6]? The sequential steps used to partially purify and lyophilize coagulation factor concentrates decrease the infectivity of HIV and other retroviruses by 1 or more logs [7, 8]. Moreover, this possibility may explain the variability in HIV symptoms and the apparently low rate of progression to AIDS within the HIV-seropositive hemophilic population [1] (summarized in [6]). If some HIV-seropositive men are not infected with HIV, they could provide valuable information concerning the effects of vaccination with HIV viral antigens.

Second, why did some persons exposed to coagulation factor products between 1980 and 1984 remain HIV-seronegative? In documented instances of exposure to known contaminated lots, not all exposed persons seroconverted [9, 10]. Exposed but seronegative individuals could possibly be infected with HIV despite their lack of antibody production: For example, persons who seroconvert while using only donor-screened, virus-inactivated coagulation factor products may have been previously infected by

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non-virus-inactivated products [11, 12] (unpublished data). HIV virus positivity in seronegative, clinically well persons is rare [13–16] but might be more common in HIV-seronegative recipients of non-virus-inactivated blood factor concentrates.

Third, are any HIV-seronegative sex partners of HIV-infected hemophilic men infected with HIV? Although many such couples have frequent, unprotected sexual intercourse, the HIV seroprevalence rate for sex partners of seropositive hemophilic men continues to be a relatively low 10%–20% [17–19]. Also, in some instances the sex partners of either homosexual or hemophilic men remained healthy and HIV-seronegative but culture-positive [15, 20, 21]. In a recent study, 4 of 16 seronegative sex partners of seropositive persons had HIV genetic material in the DNA of their peripheral blood mononuclear cells (PBMCs); however, information concerning participant selection and characteristics was not provided [22]. Thus the frequency of a seronegative virus-positive state in sex partners of HIV-infected men remains to be resolved.

Participants and Methods

We tested PBMCs from participants in two longitudinal studies: 28 factor VIII concentrate recipients and 40 factor IX concentrate recipients who were enrolled in a cohort study in 1984 and followed at 12- to 18-mo intervals thereafter [9] and 25 seropositive husband–seronegative wife pairs enrolled in a hemophilic household study in which participants were asked to return yearly [17, 19]. The cohort study participants included 20 persons who were HIV-seropositive and 48 who were seronegative at enrollment; half had received coagulation factor from lots known to contain plasma from a donor who later developed AIDS. The men from hemophilic households included 6 with AIDS, 4 with persistent generalized lymphadenopathy, 3 with HIV-related constitutional disease or secondary non-AIDS infections, and 12 without HIV-related symptoms [23]. Six seropositive asymptomatic men were enrolled in both studies; thus the total of seropositive individuals was 40.

PBMCs and DNA were isolated from participants and subjected to PCR as previously described [4], with some modifications. PBMCs were lysed 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, 1 μg of gelatin/ml, and 100 μg of proteinase K/ml. The DNA preparation was incubated at 56°C for 1 h and then at 95°C for 10 min. PBMC DNA (1 μg, representing DNA from 150,000 cells) was subjected to 35 rounds of amplification and detection using primer pair SK38/29 and ^{32}P-labeled SK19 probe as previously described [4, 24, 25]. All testing with this primer pair was done without knowledge of other laboratory results or clinical findings. On the basis of experiments in which serially diluted HIV-1 plasmid DNAs of known copy numbers (0–10^5) were spiked with 1 μg of DNA from a seronegative person and were amplified in the same manner as for DNA from unknown specimens, we estimate that with this primer pair we can detect as few as 20 viral copies per 150,000 cells.

A second primer pair, CO71 (TGTGGAGGGAATTCTTCTACTGTAA) and CO72 (TATAGAATTCACTTCTCCAATTGTCCCTCAT), designed for the amplification of a 320-base pair (bp) segment of the *env* gene of HIV-1, was used for the DNA of one seronegative PCR-positive participant and for seven seropositive persons who were PCR-negative with the first primer pair. Amplified DNA (10% of the amplification products, 10 μl) was electrophoresed in a 5% polyacrylamide gel, transferred onto a nitrocellulose filter, and hybridized with a solution containing 10 m*M* Tris HCl (pH 7.6), 0.9 *M* NaCl, 6 m*M* EDTA, 0.25% (wt/vol) nonfat dry milk, and ^{32}P-labeled CO75 oligomer (CAAATATTACAGGGCTGCTATTAACAAGAGATGGTGGTAA) at 37°C for 1 h. The filter was washed three times, 10 min each, at 37°C with a solution containing 2× SSC (30 m*M* NaCl and 3 m*M* sodium acetate) and 0.1% sodium dodecyl sulfate (SDS). The filter was exposed to Kodak X-OMAT film (Rochester, NY) at −70°C for 16 h.

Simultaneously collected serum specimens were tested for antibody to HIV by Western blot analysis [9]. Serologic reactions with the 41-kilodalton (kDa) protein of HIV or with the 25-kDa protein in association with a reaction to any other HIV protein (18-, 53-, 65-, or 110-kDa) were scored as positive; lack of reactivity to the 18-, 25-, and 41-kDa HIV proteins was scored as negative. Ninety-five of the samples were also tested for HIV antigen with the Abbott (North Chicago) enzyme immunoassay; results were recorded as negative, positive, or indeterminate.

Results

The relation between PCR results and HIV antibody and antigen status for hemophilic men in both studies is shown in table 1. The rate of concordance

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Table 1. Results of polymerase chain reaction (PCR) detection of HIV-1 DNA in HIV-seropositive and -seronegative hemophilic men.

HIV antibody/antigen status (*n*)	PCR results		
	Positive	Indeterminate	Negative
Positive			
Positive (4)	4 (100)	0	0
Indeterminate (7)	7 (100)	0	0
Negative (23)	22 (95.7)*	1 (4.3)	0
Untested (6)	5 (83.3)*	0	1 (16.7)
Total† (40)	38 (95.0)	1 (2.5)	1 (2.5)
Negative			
Positive (0)	0		0
Indeterminate (1)	1 (100)		0
Negative (45)	1 (2)		44 (98)
Untested (1)	0		1 (100)
Total‡ (47)	2 (4)		45 (96)

NOTE. Data are given as number (%).

* Four seropositive antigen-negative and one seropositive antigen-untested participants were PCR-negative with primer pair SK38/39 but PCR-positive with primer pair CO71/72.

† Six seropositive hemophilic household study participants were not tested for HIV antigen.

‡ One seronegative cohort study participant was not tested for HIV antigen.

between antibody status and PCR results did not differ for cohort study and household study participants and was 95% (83/87) overall.

Seropositive hemophilic men. Thirty-three (83%) seropositive men were PCR-positive with primer pair SK38/39 and seven were negative. Of these, five were PCR-positive with primer pair CO71/72, one was indeterminate, and one was negative; all used factor VIII concentrates between semi- and bimonthly and two, including the one who was PCR-indeterminate, were cohort members who received products from lots thought to be contaminated with HIV [9].

Subsequent PBMC samples were available for PCR assessment from two of the five men positive with the second primer pair; one of these was PCR-positive with primer pair SK38/39. An earlier sample from another of the five was also PCR-positive with this primer pair. The PCR-indeterminate and PCR-negative individuals would not provide additional blood samples; no cells remained from either person's initial blood sample for either cocultivation or DNA extraction.

None of the seven who were PCR-negative with the first primer pair had symptoms of HIV infection at the time PBMCs were drawn for PCR testing; one of the five who were PCR-positive with the second primer pair had HIV-related symptoms [23] at his latest evaluation (in 1988 for four persons, in 1987 for the other). In January 1989, the PCR-indeterminate individual was healthy but had mild axillary lymphadenopathy and lingual leukoplakia; the PCR-negative individual was also healthy. Symptomatology in the seven PCR-negative with the first pair did not differ significantly from that of the 33 who were PCR-positive with primer pair SK38/39: 8 had AIDS and 4 had other HIV-related symptoms at the time PBMCs were drawn for PCR testing, and 11 had AIDS and 3 had other HIV-related symptoms at the latest evaluation. There were no obvious differences by PCR status in numbers of T helper lymphocytes [T(h)] or ratios of T helper to T suppressor [T(s)] cells.

Seronegative hemophilic men. No persons seronegative while using only donor-screened, virus-inactivated concentrates ($n = 41$) were PCR-positive. Two others who were still using non-virus-inactivated products at the time of testing were PCR-positive. Both were tested for the presence of HIV antigen; one was antigen-negative and one was antigen-indeterminate. Both were frequent users of factor VIII concentrate and recipients of products from suspected contaminated lots [9].

The antigen-indeterminate person, a 22-y-old, had detectable HIV antibodies at his next evaluation (11 mo later) but was in good health as of August 1988. A second PBMC sample drawn after he had seroconverted was PCR-positive with primer pair SK38/39. His T(h) count was 618/mm³ and T(h):T(s) ratio was 0.4 at initial testing and 576/mm³ and 0.6, respectively, after seroconversion.

The PCR-positive antigen-negative person was 66 y old at initial evaluation and died of a cerebrovascular accident before his next scheduled evaluation. His T(h) count was 266/mm³ and T(h):T(s) ratio was 0.5. An independently processed vial of PBMCs obtained on the same data as the first sample was also PCR-positive with primer pair SK38/39 and with primer pair CO71/72.

Couples. No seronegative sex partners of seropositive hemophilic men were PCR-positive, irrespective of the hemophiliac's HIV-related health status. Five (28%) of 18 husbands and 0 of 15 wives tested for HIV antigen were positive or indeterminate. Of the 25 couples, 24 still had vaginal intercourse at the time PBMCs were drawn; 12 also reported having oral intercourse. One couple had discontinued sexual activity in 1981, 1 had intercourse less than once a month, 20 had intercourse two or more times a month, and 3 were not asked to specify frequency.

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Sixteen specified having unprotected intercourse, 5 used condoms 10%–100% of the time, and 4 were not asked to specify condom usage.

Discussion

We used a relatively new technique, PCR, to search for HIV-1 DNA sequences in PBMCs of hemophilic men and their sex partners [4, 5, 25]. Three questions were addressed.

First, were seropostive hemophilic men also PCR-positive? Most (83%) were indeed PCR-positive by use of primer pair for the *gag* region; however, 7 were PCR-negative with this primer pair. Five of these were PCR-positive with a second primer pair for the *env* region, one was PCR-indeterminate, and one was PCR-negative.

These discrepancies between primer pairs were probably due to the limits of PCR sensitivity (i.e., too few cells were infected or too few HIV DNA copies were present to be detected with the first primer pair) rather than to a true absence of HIV infection and a false-positive reaction with the second primer pair because: At least two of these persons received contaminated product [9]; all seven received coagulation factor concentrates during a period of widespread contamination [1, 2]; all received factor VIII products, which are more strongly associated with HIV seroconversion and AIDS than are factor IX products [26]; one of two tested was PCR-positive with the first primer pair on a subsequent sample; one was PCR-positive with the first primer pair on an earlier sample; and another later developed symptoms of HIV infection.

The longitudinal design of the cohort study will permit us to follow the two who were not PCR-positive with either primer pair to determine whether they might be not infected with HIV but "immunized" with inactivated virus [6] and to clinically evaluate the possibility that some PCR-positive persons were infected with defective HIV-1. However, the present data suggest these possibilities are doubtful and, indeed, that all seropositive hemophilic persons should consider themselves infected.

Second, were any seronegative asymptomatic hemophilic men positive by PCR testing for HIV-1 DNA? No persons who remained seronegative in 1985 while receiving only donor-screened, virus-inactivated coagulation factor products were PCR-positive, consistent with other data on the safety of these products in terms of HIV. Two of six seronegative individuals tested while still on unscreened, non-treated products were PCR-positive; one seroconverted within 11 mo and the other's death from non-HIV-related causes precluded further assessment. Additional PCR testing, including the use of a second primer pair on one, substantiated that these persons were HIV-1 DNA–positive. These results support the relative safety of donor-screened, virus-inactivated coagulation factor products and indicate that HIV-1 DNA can sometimes be detected before seroconversion.

Third, were any seronegative sex partners of seropositive hemophilic men positive by PCR for HIV-1 DNA? The rates of seropositivity for sex partners of hemophilic men remain at 10%–20% [17–19]. Moreover, HIV or HIV DNA has been isolated from HIV-seronegative sex partners of HIV-infected men [15, 20–22]. We assessed 25 couples who had vaginal intercouse and in which the men had a full range of HIV symptoms. No woman was positive by PCR, but our sample size permits only a 16.6% upper limit to the 95% confidence interval for the possible rate of infection in seronegative sex partners of hemophilic men [27]. Also, this finding by no means indicates that sex partners of HIV-infected men are at low risk of HIV infection. However, it should be extremely reassuring for seronegative women no longer having unprotected intercourse with HIV-infected hemophilic men.

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