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Absence of reproducibly detectable low-level HIV viremia in highly exposed seronegative men and women.

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Abstract

Objective—Transient HIV infections have been invoked to account for the cellular immune responses detected in highly virus-exposed individuals who have remained HIV seronegative. We tested for very low levels of HIV RNA in 524 seronegative plasma samples from 311 highly exposed women and men from 3 longitudinal HIV cohorts.

Design—2073 transcription mediated amplification (TMA) HIV RNA tests were performed for an average of 3.95 TMA assays per plasma sample. Quadruplicate TMA assays, analyzing a total of 2 ml of plasma, provided an estimated sensitivity of 3.5 HIV RNA copies/ml.

Results—Four samples from subjects who did not sero-convert within the following six months were positive for HIV RNA. For one sample, human polymorphism DNA analysis indicated a sample mix up. Borderline HIV RNA detection signals were detected for the other three positive samples and further replicate TMA testing yielded no positive results. Nested PCR assays (n=254) for HIV proviral DNA on PBMC from these 3 subjects were negative.

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Conclusions—Transient viremia was not reproducibly detected in highly HIV exposed seronegative men and women. If transient infections do occur, plasma HIV RNA levels may remain below the detection limits of the sensitive assay used here, be of very short duration, or viral replication may be restricted to mucosal surfaces or their draining lymphoid tissues.

Introduction

Anti-HIV cellular immune responses have been reported in some highly HIV exposed but consistently seronegative individuals leading to the possibility that particularly effective early cellular immunity might, on occasion, eliminate HIV infections [1-8]. The presence of HIV viral nucleic acids was also reported in blood collected over 12 months prior to seroconversion [9, 10] and very low levels of HIV proviral DNA were reported in two highly HIV exposed, consistently seronegative male homosexuals [11]. Low dose SIV mucosal challenges in macaques have also lead to apparently transient low-level viremia months prior to the establishment of chronic viremia and seroconversion [12-16]. The continued presence of very low levels of SIV proviral DNA in PBMCs years following inoculation in seronegative macaques suggests a smoldering infection [17]. Transient or persistent low-level HIV or SIV replication in the absence of seroconversion might therefore reflect particularly effective innate or cellular immune responses, infections with highly attenuated HIV strains, and/or host genetic restrictions on sustained viral replication.

Detailed investigations of transient viremia reported in newborns indicated a lack of satisfactory documentation for these infections [19, 20]. Some of the highly exposed seronegative subjects with detectable cellular immune response were subsequently shown to undergo primary HIV infections with seroconversion, indicating against a long-term protective impact [2, 21]. To test for the occurrence of transient viremia we tested seronegative plasma samples from highly exposed individuals in 3 HIV infection cohorts for very low levels of HIV RNA. PBMC from potentially viremic cases were then analyzed for proviral HIV DNA.

MATERIAL AND METHODS

San Francisco Men's Health Study (SFMHS) [22]

All tests were approved by the UCSF committee on human research. The SFMHS was a prospective study of high-risk gay men sampled from 1985 to 1995. Seronegative SFMHS participants were identified at the time points when they reported engaging in receptive anal intercourse with the greatest number of partners (> 3 insertive partners or >1 insertive partner without condom in prior months). Plasma from seroconverters were also selected for analysis at serial time points before and at seroconversion.

San Francisco City Clinic Cohort (SFCCC) [23, 24]

This longitudinal cohort of gay and bisexual men treated for sexually transmitted infections at the San Francisco City Clinic was initiated in 1978. We identified samples from time-points between 1983-1985 from men who reported the highest risk behavior as determined by a risk score incorporating number of partners and proportion of sex acts involving unprotected anal receptive sex with ejaculation in the past 4 months.

Women Interagency Health Study (WIHS) [25]

Plasma samples from seronegative women with the most exposure to HIV (sex with known HIV infected partner, number of partners, unprotected anal sex) were tested.

TMA assay

The primary assay used for the detection of low level HIV RNA was the isothermal Transcription Mediated Amplification or TMA (APTIMA HIV RNA qualitative assay, Gen-Probe San Diego, CA). The TMA assay was FDA approved as a qualitative test to screen blood donations for HIV RNA and has been approved for clinical use. TMA has a sensitivity of 5.0 copies RNA/ml (50% limit of detection) or 13 copies RNA/ml (95% limit of detection) when testing 0.5 ml of plasma [26-28]. When plasma volume was sufficient we tested multiple 0.5 ml aliquots per time point (average 3.95, range 2 to 6). The use of TMA in quadruplicate assay (using 4 × 0.5 ml plasma) improved the limit of detection to <3.5 HIV RNA copies/ml [29]. A TMA signal/cutoff (S/CO) ratio >1.0 is considered a positive signal. At low viral loads a direct relationship has been observed between rising S/CO ratios from 1.0 to 20 and rising viral loads [29]. Above 100 HIV RNA/ml the S/CO is saturated [29].

Human genetic polymorphisms test

Twelve PCR assays targeting polymorphic human chromosome sites where insertion/deletion differences are frequently observed were used as described [30].

Proviral DNA nested PCR (nPCR)

Genomic DNA was extracted from thawed frozen PBMC using the QIAamp DNA blood kit (Qiagen). A nPCR targeting the HIV gag p17 region was used with 400ng of PBMC genomic DNA as input. The PCR primers and conditions are as described except that primer PG6 was replaced by 5'actttaccatgcatttaaagttctaggt3' [11]. Using plasmid pNL43 dilutions containing 3, 10, 50, 100 copies as input, the nested PCR was performed in quadruplicates. The sensitivity of the nPCR was estimated using agarose gel electrophoresis at 3-10 HIV DNA copies in the presence of 1ug of genomic DNA.

RESULTS AND DISCUSSION

HIV RNA testing of plasma from 3 cohorts

A total of 196 seronegative plasma samples from 133 subjects (122 non-seroconverters and 11 seroconverters) from the SFMHS were tested for low-level HIV RNA using 921 TMA assays (Table 1). Additionally, nine HIV antibody positive plasma samples from subjects who had seroconverted within the previous six months (i.e., prior bleed was seronegative) were also tested. The nine seropositive plasma samples were all strongly HIV RNA positive, with all TMA replicate tests yielding S/CO values of > 15. Plasma samples from eleven seronegative subjects who seroconverted during the following six months (i.e., were seropositive at the subsequent bleed) were included among the 196 seronegative samples. Two of these eleven subjects were found to be in their primary HIV infection phase with similarly strong HIV RNA positive signals in all TMA replicate assays. In contrast none of the 25 samples collected more than six months prior to seroconversion in these 11 subjects were viremic.

The 122 highly exposed non-seroconverters from the SFMHS were also tested using multiple replicate TMA tests per sample. All samples except one (ID 50320) were TMA negative in all replicate assays (S/CO<1). A single sample had one out of five TMA replicate assays with a positive S/CO of 2.0. In order to further assess the viremic nature of this sample collected in February 1986, 8 ml of additional plasma from the same subject and time point was tested using 16 TMA replicate assays. All 16 replicate TMA assays were negative.

Of the 42 seronegative plasma samples provided from 42 SFCCC participants tested using 109 replicate TMA assays only one was HIV RNA positive (SFCCC 5093) (Table 1). This sample was strongly positive with all four TMA replicate positive with S/CO>15. A new aliquot of this plasma sample was acquired from the SFCCC repository. The sample again tested strongly HIV RNA positive in 2/2 TMA replicates. The sample was collected in August 1984, from a subject who remained seronegative at all four subsequent time points (March 1985, October 1985, February 1987, and April 1988), but was seropositive in March 1989 and at five subsequent time points through August 1990. In order to rule out any sample mix-up we analyzed 12 highly polymorphic human DNA alleles [30] from the August 1984 plasma sample, PBMC DNA from the same time point, and PBMC DNA from both a February 1987 and a February 1990 clinic visit. The August 1984 plasma and PBMC human DNA matched at all 12 polymorphic alleles, but only 2/12 loci matched with the 1987 and 1990 PBMC samples. The 1987 and 1990 samples matched at all 12 loci. These results indicated that the 1984 plasma and PBMC came from a different person than the 1987 and 1990 samples. We concluded that the strongly HIV RNA positive 1984 plasma sample could not be assigned to the individual who sero-converted between April 1988 and March 1989, and represented a sample mix up from a viremic individual. PBMC derived DNA from the 1984 collected specimen was also tested for HIV proviral DNA, and found to be HIV DNA positive.

A total of 136 seronegative women in the WIHS with the most exposure to HIV were selected. Two hundreds and eighty-six plasma samples from the time points when exposure was highest for these subjects were then tested using 1043 TMA assays (Table 1). Seven of 286 plasma samples yielded positive TMA test results. Five of the positive plasma samples were from the time points collected immediately prior to seroconversion, and therefore represented typical primary HIV infection (in these 5 cases 4/4 replicate TMA were strongly positive with S/CO>15). The other two samples (WIHS 60101030 and WIHS 20204650) were from women who did not seroconvert at the subsequent bleed. These two samples had very low-level HIV RNA reactivity, in both cases only one out of four replicate TMA assays were positive with positive yet low S/CO of 1 to 2. Further plasma aliquots from these two subjects at the same time points were acquired and re-analyzed by replicate TMA assays. None of four replicate TMA tests run on each of these two samples were positive.

Negative proviral DNA testing of cases with possible transient viremia

In order to test for the presence of proviral DNA in the 3 potentially transiently infected subjects (SFMHS 50320, WIHS 20204650, WIHS 60101030), we used a nested PCR protocol targeting the gag region.

PBMC were not available from the same time point as the weakly TMA positive 1986 plasma sample from SFMHS subject 50320. PBMC collected at two time points in 1989, when the subject was still seronegative, were therefore selected for HIV proviral DNA testing. Ten repeat nPCR tests were performed on PBMC DNA from both 1989 samples. None of the 20 nPCRs were positive (Table 1).

The second sample showing minimal positive evidence of HIV viremia was from WIHS subject 60101030 and had been collected in August, 1999. DNA was extracted from PBMC collected from this time point as well as on March, 1999, February, 2000, and November, 2005 and a total of 36, 17, 35, and 7 nPCRs were performed. No proviral HIV DNA was detected.

The third sample showing possible evidence of HIV viremia was collected from WIHS subject 20204650 on February, 1999. DNA was extracted from PBMC collected on that date as well as September, 1999, February, 2000 and January, 2006 and a total of 33, 41, 57, and

8 nPCR for proviral DNA were performed. No proviral HIV DNA was detected. Proviral HIV DNA was therefore not detected in the PBMC from the same or later time points from any of the subjects with possible transient viremia.

From a total of 524 seronegative plasma samples collected from 311 highly exposed men and women, only 4 samples showed any evidence of HIV RNA more than six months prior to seroconversion. One case was the consequence of a sample mix up. In the remaining three cases only a single out of four or five replicate TMA assays were positive and then only with very low-level S/CO ratios. Further replicate TMA testing of more plasma failed to yield any positive results. Because very low levels HIV proviral DNA might represent an archive of a past transient infection [11, 17] we tested these 3 subjects for proviral DNA using nPCR. No PBMC proviral reservoirs were detected.

Our negative results indicate that if transient viremia occurs, it must be either at levels too low to be reproducibly detected and/or of too short duration to have been sampled in the plasma tested here. It also remains conceivable that viral replication is restricted near mucosal surfaces or in draining lymphoid tissues and therefore not able to reach detectable plasma levels [31-34]. Alternatively the cellular immune responses reported in highly exposed seronegatives may be due to their continued exposure to non-infectious HIV proteins rather than to viral replication.

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HIV cohort	Male subjects analyzed	Female subjects analyzed	plasma samples tested	TMA tests performed	Potential transient viremia	Transient infection confirmed by
SFMHS	133	0	196	921	1	0/1
SFCC	42	0	42	109	1	0/1 (Sample mix up)
WIHS	0	136	286	1043	2	0/2
Total	175	136	524	2073	4	0/4