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Molecular analysis of HIV strains from a cluster of worker infections in the adult film industry, Los Angeles 2004

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Abstract

Objectives: In April 2004, 13 susceptible women were exposed to a single acutely HIV-1-infected man while employed to perform various sex acts for the production of adult films; three women were subsequently found to have acquired HIV infection (23% attack rate). As part of the investigation of this infection cluster, we evaluated whether viral strains collected from infected individuals were significantly related.

Methods: We determined nucleotide sequences from the C2V3C3 and gp41 region of *env* and the p17 region of *gag* in viruses from the three infected individuals from whom specimens were available. We then compared these sequences phylogenetically to comparable sequences from available reference strains. Genotypic and phenotypic antiretroviral drug resistance was determined for plasma virus from the male index case and one female contact at a separate commercial laboratory.

Results: The *env* and *gag* sequences of the HIV strains from the male index case and two of the infected women were 100% similar. Genotyping of the male index case's virus identified 12 mutations, which represented known naturally occurring polymorphisms in the subtype B consensus sequence that are not associated with antiretroviral drug resistance. Genotyping of the virus from the female contact identified 10 mutations, all of which were shared by the virus from

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Sequence data

The nucleotide sequence data for the C2V3C3 region and partial gp41 of *env* and the p17-coding region of *gag* were deposited in the GenBank nucleotide sequence database (accession numbers [DQ148506–DQ148514](#)).

the male index case. Phenotyping demonstrated that both viruses were susceptible to all antiretroviral drugs tested.

Conclusion: Molecular and virological data strongly support the epidemiological conclusion that these women were infected with an identical strain of HIV through occupational exposure to an individual with an acute HIV infection.

Keywords

Adult film industry; HIV-1; infection cluster; Los Angeles; sequence analysis; viral drug resistance

Introduction

Molecular sequence analysis of HIV strains collected from exposed individuals during cluster investigations can provide important data in support of epidemiological evidence regarding transmission patterns. Earlier investigations of potentially related HIV infections have used genetic and phylogenetic techniques to provide data to support or refute person-to-person transmission of HIV in healthcare settings [1–3], within households [4], between sexual partners [5,6], and in cases of intentional infection through injection [7].

In April 2004, a cluster of four new HIV infections was identified among performers in the adult film industry in Los Angeles County. Epidemiological investigation, reported elsewhere [8], indicated that a male index case had infected three of 13 women (23% attack rate) with whom he had been employed to perform a variety of sexual acts in the production of adult films. These acts included oral, vaginal, anal, and double-anal (two penises in one woman's anus) sex. All sex acts were performed without barrier precautions (e.g. condoms). The exposures took place during a 26-day period after the male index tested HIV negative (17 March 2004) and then HIV positive (9 April 2004) using a polymerase chain reaction (PCR) DNA-based method that detects proviral DNA (Amplicor HIV-1 test; Roche Molecular Systems, Branchburg, New Jersey, USA). This test is approved for research use only and is labeled specifically not for use in diagnostic procedures, but was used in this context to reduce the window period between HIV infection and its diagnosis; PCR-based methods are estimated to reduce this window period to approximately 10 days [9].

The three HIV-infected female performers all tested HIV negative before working with the male index case and then HIV positive after this work (Fig. 1). The index case and two of the women reported no other sexual contacts. The sole non-occupational contact of the third woman (contact no. 3, Fig. 1) tested HIV negative 60 days after her last potential sexual exposure to HIV. The epidemiological investigation of this infection cluster concluded that the three women were infected through occupational exposure. We evaluated whether the available viral strains collected from infected individuals were related and supported this epidemiological conclusion.

Methods

Blood specimens were available from the male index case and two of his three infected female partners (contacts nos. 1 and 2, Fig. 1). Specimens from consenting individuals were

collected within 3 months after the diagnosis of HIV infection before any antiretroviral exposure, and were evaluated at the Centers for Disease Control and Prevention for relatedness by RNA extraction, reverse transcriptase (RT)–PCR amplification, direct or consensus DNA sequencing, and genetic analysis. HIV RNA was extracted from blood plasma using the Qiagen UltraSens extraction kit (Qiagen Inc., Valencia, California, USA). Extracted RNA was used to amplify the C2V3C3 and gp41 regions of *env* [10–12] and the p17 region of *gag* [13]. These regions have been used for phylogenetic comparisons of HIV strains in transmission cases because they are located in separated portions of the HIV genome, and encode proteins with different functions subject to generally independent selection pressures related to the host immune response and transmission. The Qiagen One-Step RT–PCR kit was used to amplify various amounts of template RNA. Amplification conditions were as follows: 30 min at 50°C, 15 min at 95°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C (50°C for gp41 amplification), and 1 min at 72°C. Secondary amplification using 1–5 µl of primary reactions was performed using the Platinum Taq PCR Supermix (Invitrogen Corp., Carlsbad, California, USA), with the same thermocycling conditions as in the primary amplification but without the 30 min 50°C step and 15 min 95°C step. Secondary PCR amplicons were purified using QIAquick PCR purification kits (Qiagen Inc.) and were subsequently sequenced with the nested amplification primers using the BigDye Terminator version 1.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA) and an automated ABI Prism 3100 DNA sequencer (Applied Biosystems). The HIV regions sequenced included the C2V3C3 region (454 nucleotides) of *env*, a portion of the gp41 coding region (354 nucleotides) of *env*, and the p17-coding region (396 nucleotides) of *gag*; these regions are used commonly in phylogenetic analyses and comparisons of HIV strains in transmission cases. Sequences were edited using Sequencher version 4.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and aligned using the Se-Al Sequence Alignment Editor, version 1.0 [14].

To reduce the possibility of cross-contamination between samples during these analyses, RNA from each of the three blood specimens was extracted by a single laboratory scientist on different days. All subsequent amplification and sequencing of the extracted RNA was performed in duplicate by two different laboratory scientists on separate days in physically separated laboratories.

The C2V3C3, gp41, and p17 sequences from each case patient were compared with the other case patients' sequences. These sequences were then compared with equivalent sequences from HIV reference strains collected in geographical regions where the HIV transmissions occurred: California and Brazil. Reference sequences were sought by searching the HIV Sequence Database (Los Alamos National Laboratories, New Mexico, USA); the number of available reference sequences for C2V3C3, gp41, and p17 of equivalent length to the sequences from the case patients that also had adequate information on the geographical location of the collection source was limited. For these reasons, and because individuals working in the adult film industry to which the case patients were exposed often work in other US cities, we expanded the search from California and Brazil to also include sequences from any other location in the United States. We identified 17 US sequences for C2V3C3, nine Brazil sequences for V3 alone, 18 US sequences for gp41, and two US and three Brazil sequences for p17. In this database we identified no equivalent

sequences from the United States that were specified as being from California. Additional comparison data were taken from unpublished sequences of HIV-1 subtype B strains previously analysed at CDC: 11 California sequences for C2V3C3, 15 non-California US sequences for gp41, and 11 California and 10 non-California US sequences for p17. All alignments were examined both visually and by computer, using MEGA, version 3.0 [15] for differences in nucleotide sequences and to determine nucleotide distances (mean substitutions per 100 sites $\pm$ standard error).

The C2V3C3, gp41, and p17 sequences from the case patients, other identified reference sequences from HIV-1 subtype B strains, as well as representative sequences from the other eight HIV-1 subtypes were used in three separate alignments as input for phylogenetic analysis. Appropriate nucleotide substitution models were determined with Modeltest v3.6 [16] for each of the three alignments and bootstrap (2000 replicates), neighbor-joining trees generated with PAUP* v4.0 [17].

Two case patient's viral isolates (the index case and contact 1, Fig. 1) were also tested for antiretroviral resistance mutations by both genotyping and phenotyping at a commercial laboratory (ViroLogic PhenoSense GT, South San Francisco, CA, USA). These specimens were collected and assayed before any antiretroviral exposure.

Results

The three case patient HIV-1 strains were determined to be subtype B. The results of within-group and between-group comparisons are shown in Table 1 and Table 2. The three case patient isolates were identical to each other, whereas a within-group comparison of sequences from reference strains varied from 8.2 ± 0.8 to 15.5 ± 1.1 substitutions per 100 nucleotides among comparisons of all three gene regions. Between-group comparisons of the case patients' sequence group with equivalent sequences from reference strain groups showed a similar variation: 8.0 ± 1.0 to 19.6 ± 2.3 substitutions per 100 nucleotides. As expected, phylogenetic analysis indicated that the case patients' sequences clustered strongly (100% bootstrap support) with HIV-1 subtype B strains within the reference HIV subtype phylogenetic trees, but not with other strains in the subtype B trees; a representative phylogenetic tree for the C2V3C3 gene region on *env* is shown in Fig. 2.

There was no evidence of genotypic or phenotypic antiretroviral drug resistance in the plasma virus tested from the male index case and his female contact. For the male index case, the only differences from subtype B consensus sequence in RT were at codons Q102K, I142M, C162S, R211K, F214L, A272T, V276I and R277K. Plasma virus from the female partner had identical differences but lacked detection of the mixtures seen at codons 272 and 276. Mutational differences seen in protease included I15V, N37S, R41K and V77I, which were also identical in both individuals. All of the mutations identified reflected naturally occurring poly-morphisms that are not associated with antiretroviral drug resistance.

Discussion

Our analysis demonstrates that the HIV-1 strains from the three case patients represent a cluster of viruses highly related to one another, but unlike the limited available comparison

strains to which the case patients could have been exposed based on geography. Two lines of evidence support a close molecular relationship between the HIV strains examined: data from the genetic sequencing performed at the Centers for Disease Control and Prevention laboratory and the genotypic and phenotypic resistance data obtained at a commercial laboratory. Although our analysis was limited to HIV strains from three of the four HIV-1 infections in the identified cluster and by the small number of reference sequences available for comparison, the molecular data support the epidemiological evidence of occupational HIV transmission.

The finding that the case patients' viruses shared identical nucleotide sequences at the evaluated gene loci, in one case 3 months after transmission, is unusual but not unexpected. The male index case transmitted HIV during the primary acute phase of his infection, the women were infected by the index case within a few days of one another, and blood specimens for molecular and microbiological analyses were collected before exposure to selective drug pressure. Investigations of other well-characterized HIV infection clusters have also found identical sequences in strains collected 3 months apart from individuals with epidemiologically related infections [18–20]. The high rate of spontaneous mutation of the virus can produce a variety of quasispecies within a single infected individual. The methods used in this and other similar investigations as well as in clinical HIV medicine detect the gene sequences predominating in the population of circulating virions; these methods are widely accepted for identifying mutations. The development of fixed mutations in the population of virions leading to detectable genetic divergence is slow compared with the rate of change within individual virus particles as they reproduce. Quantifying the number of quasispecies as a means of documenting mutational variation is labor-intensive, costly, and generally considered impractical.

The 23% attack rate in this cluster of infections was considerably higher than the most conservatively estimated risk of less than 0.5% for a single act of receptive anal or receptive vaginal sex with an HIV-infected partner [21,22]. The HIV transmission risk for the presently investigated cluster was greater for at least three reasons. First, the sexual contact involved in adult film production can be prolonged and traumatic, increasing the opportunity for infection to occur; notably in the case of these infections there was a substantially increased risk of trauma to the anorectum (i.e. double-anal penetration). Second, most estimates of transmission risk are 'per coital act' calculations. The risk for adult film workers is increased by their multiple coital and other exposures over a short period; the attack rate for this cluster represents a cumulative rather than per-act risk. Finally, the transmissibility of HIV is greatest within the first months after infection during and near the time of seroconversion. Epidemiological studies in Africa have observed a 12-fold higher risk per coital act for heterosexual transmission within the first 5 months after initial infection [23]. Mathematical models using US data estimate that the higher loads of HIV in semen observed during seroconversion increase the risk eight to 10-fold that a man acutely infected with HIV and free of other sexually transmitted diseases (STD) will transmit the virus to his female partner, infecting 7–24% of susceptible female partners during the first 2 months after infection [24].

Routine frequent HIV testing can identify individuals during the acute primary phase of HIV infection when they are most infectious to others. Such testing should be encouraged, particularly among individuals at a substantially increased risk of exposure to HIV, such as adult film workers. HIV testing should be coordinated with STD screening when there is any risk of co-infection, especially since STD infections increase the risk of both HIV transmission and acquisition. Routine testing is important for early diagnosis and referral for care. However, as this cluster of occupational HIV infections illustrates, testing alone, even by the most sensitive available approaches, is an inadequate prevention strategy. Condoms and other barrier methods should be used consistently during filming to reduce occupational exposure to infected fluids and prevent the transmission of HIV and STD between workers.

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References

1. Ou CY, Ciesielski CA, Myers G, Bandea CI, Luo CC, Korber BT, et al. Molecular epidemiology of HIV transmission in a dental practice [Comment]. *Science* 1992; 256:1165–1171. [PubMed: 1589796]
2. Holmes EC, Zhang LQ, Simmonds P, Rogers AS, Brown AJ. Molecular investigation of human immunodeficiency virus (HIV) infection in a patient of an HIV-infected surgeon. *J Infect Dis* 1993; 167:1411–1414. [PubMed: 8501332]
3. Jaffe HW, McCurdy JM, Kalish ML, Liberti T, Metellus G, Bowman BH, et al. Lack of HIV transmission in the practice of a dentist with AIDS. *Ann Intern Med* 1994; 121:855–859. [PubMed: 7978698]
4. Centers for Disease Control and Prevention. Human immuno-deficiency virus transmission in household settings – United States. *MMWR Morb Mortal Wkly Rep* 1994; 43:347–356. [PubMed: 8177193]
5. Centers for Disease Control and Prevention. Cluster of HIV-positive young women – New York 1997–1998. *MMWR Morb Mortal Wkly Rep* 1999; 48:413–416. [PubMed: 10365630]
6. Robbins KE, Weidle PJ, Brown TM, Saekhou AM, Coles B, Holmberg SD, et al. Molecular analysis in support of an investigation of a cluster of HIV-1-infected women. *AIDS Res Human Retroviruses* 2002; 18:1157–1161. [PubMed: 12402955]
7. Veenstra J, Schuurman R, Cornelissen M, van't Wout AB, Boucher CA, Schuitemaker H, et al. Transmission of zidovudine-resistant human immunodeficiency virus type 1 variants following deliberate injection of blood from a patient with AIDS: characteristics and natural history of the virus. *Clin Infect Dis* 1995; 21:556–560. [PubMed: 8527543]
8. HIV transmission in the adult film industry – Los Angeles, California, 2004. *MMWR Morb Mortal Wkly Rep* 2005; 54:923–926. [PubMed: 16177683]
9. Busch MP, Kleinman SH. Nucleic acid amplification testing of blood donors for transfusion-transmitted infectious diseases. *Transfusion (Paris)* 2000; 40:143–159.

10. Delwart EL, Shpaer EG, Louwagie J, McCutchan FE, Grez M, Rubsamen-Waigmann H, et al. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 env genes. *Science* 1993; 262:1257–1261. [PubMed: 8235655]
11. Kostrikis LG, Shin S, Ho DD. Genotyping HIV-1 and HCV strains by a combinatorial DNA melting assay (COMA). *Mol Med* 1998; 4:443–453. [PubMed: 9713823]
12. Yang C, Pieniazek D, Owen SM, Fridlund C, Nkengasong J, Mastro TD, et al. Detection of phylogenetically diverse human immunodeficiency virus type 1 groups M and O from plasma by using highly sensitive and specific generic primers. *J Clin Microbiol* 1999; 37:2581–2586. [PubMed: 10405405]
13. Schochetman GS, Subbarao S, Kalish ML. Methods for studying genetic variation of the human immunodeficiency virus (HIV). Boca Raton, Florida: CRC Press; 1996.; pp. 25–41.
14. Rambaut A Se-Al: Sequence alignment editor, version 1.0. Distributed by the author through Oxford University, Department of Zoology, Evolutionary Biology Group, Oxford, UK Available at: <http://evolve.zoo.ox.ac.uk/>. Accessed: 5 December 2005.
15. Kumar S, Tamura K, Nei M. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 2004; 5:150–163. [PubMed: 15260895]
16. Posada D, Crandall KA. Modeltest: testing the model of DNA substitution. *Bioinformatics* 1998; 14:817–818. [PubMed: 9918953]
17. Swofford D PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0. Sinauer Associates Inc. Publishers, Sunderland, Massachusetts Available at: <http://paup.csit.fsu.edu/index.html>. Accessed: 5 December 2005.
18. McNearney T, Westervelt P, Thielan BJ, Trowbridge DB, Garcia J, Whittier R, et al. Limited sequence heterogeneity among biologically distinct human immunodeficiency virus type 1 isolates from individuals involved in a clustered infectious outbreak. *Proc Natl Acad Sci USA* 1990; 87:1917–1921. [PubMed: 2308953]
19. Wolfs TFW, Zwart G, Bakker M, Goudsmit J. HIV-1 genomic RNA diversification following sexual and parenteral virus transmission. *Virology* 1992; 189:103–110. [PubMed: 1376536]
20. Ling AE, Robbins KE, Brown TE, Dunmire V, Thoe SYS, Wong SY, et al. Failure of routine HIV-1 tests in a case involving transmission with preseroconversion blood components during the infectious window period. *JAMA* 2000; 284:210–214. [PubMed: 10889595]
21. Vittinghoff E, Douglas J, Judson F, McKirnan D, MacQueen K, Buchbinder SP. Per-contact risk of human immunodeficiency virus transmission between male sexual partners. *Am J Epidemiol* 1999; 150:306–311. [PubMed: 10430236]
22. Varghese B, Maher JE, Peterman TA, Branson BM, Steketee RW. Reducing the risk of sexual HIV transmission: quantifying the per-act risk for HIV on the basis of choice of partner, sex act, and condom use. *Sex Transm Dis* 2002; 29:38–43. [PubMed: 11773877]
23. Wawer MJ, Gray RH, Sewankambo NK, Serwadda D, Li X, Laeyendecker O, et al. Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai. *Uganda J Infect Dis* 2005; 191:1403–1409. [PubMed: 15809897]
24. Pilcher CD, Tien HC, Eron JJ, Vernazza PL, Leu S, Stewart PL, et al. Brief but efficient: acute HIV infection and the sexual transmission of HIV. *J Infect Dis* 2004; 189:1785–1792. [PubMed: 15122514]

Fig. 1. Diagrammed timeline of exposures, testing and blood collection events among the index case and three HIV-infected female contacts.

| Unprotected sexual contact with index case; ⊖ HIV testing and result; ↓ blood draw for molecular analyses.

Fig. 2. Phylogenetic relationship of the HIV sequences derived from the index case and two female contacts, 11 unrelated subtype B strains from California (CA1–CA11), and 17 unrelated subtype B strains from US states other than California (US1–US17).

The tree was derived from a nucleotide alignment of the C2V3C3 region of *env* (454 nucleotides). Implementation of the neighbor-joining method with boot-strapping (2000 replicates) was done in PAUP* v4.0 [17]. The number at the node indicates the percentage of bootstrap values.

Within-group mean nucleotide distances measured as substitutions per 100 sites with estimated 95% standard of error between selected HIV-1 gene sequences of the case patient strains and equivalent sequences from various sets of reference strains.

Table 1.

Comparison strains of HIV-1 subtype B	<i>env</i>			<i>gag</i>	
	C2V3C3 (454 nucleotides)	V3 (294 nucleotides)	gp41 (354 nucleotides)	p17 (396 nucleotides)	
Case patient isolates	0.0 ± 0.0 (3)	0.0 ± 0.0 (3)	0.0 ± 0.0 (3)	0.0 ± 0.0 (3)	
California					
CDC unpublished data	15.5 ± 1.1 (11)	–	–	9.9 ± 0.9 (11)	
Other US states					
CDC unpublished data and Los Alamos database	12.5 ± 0.9 (17)	–	10.1 ± 0.9 (33)	8.2 ± 0.8 (10)	
Brazil					
Los Alamos database	–	15.6 ± 1.6 (9)	–	9.9 ± 1.4 (3)	

The number of available strains compared in each group is indicated in parentheses.

Between-group mean nucleotide distances measured as substitutions per 100 sites with estimated 95% standard of error comparing selected HIV-1 gene sequences from the case patient strains to equivalent sequences from various reference strains.

Table 2.

Comparison strains of HIV-1 subtype B	<i>env</i>			<i>gag</i>	
	C2V3C3 (454 nucleotides)	V3 (294 nucleotides)	gp41 (354 nucleotides)	p17 (396 nucleotides)	
California					
CDC unpublished data	16.1 ± 1.5 (11)	–	–	9.0 ± 1.1 (11)	
Other US states					
CDC unpublished data and Los Alamos database	14.6 ± 1.5 (17)	–	10.4 ± 1.2 (33)	8.0 ± 1.0 (10)	
Brazil					
Los Alamos database	–	19.6 ± 2.3 (9)	–	9.0 ± 0.9 (3)	

The number of available reference strains compared in each group to the case patient strains is indicated in parentheses.