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CXCR4-using HIV variants in a cohort of Black men who have sex with men: HIV Prevention Trials Network 061

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Abstract

Objective—To evaluate factors associated with HIV tropism among Black men who have sex with men (MSM) in the United States enrolled in a clinical study (HIV Prevention Trials Network 061).

Methods—HIV tropism was analyzed using a phenotypic assay (Trofile assay, Monogram Biosciences). Samples were analyzed from 43 men who were HIV infected at enrollment and reported either exclusive insertive intercourse or exclusive receptive intercourse; samples were also analyzed from 20 men who were HIV uninfected at enrollment and seroconverted during the study. Clonal analysis of individual viral variants was performed for seroconverters who had dual/mixed viruses.

Results—Dual/mixed viruses were detected in samples from 11 (26%) of the 43 HIV-infected men analyzed at the enrollment visit; HIV tropism did not differ between those reporting exclusive insertive vs. receptive intercourse. Dual/mixed viruses were also detected in five (25%) of the 20 seroconverters. Dual/mixed viruses were associated with lower CD4 cell counts. Seroconverters with dual/mixed viruses had dual-tropic viruses only or mixed populations of CCR5– and dual-tropic viruses.

Conclusions—Dual/mixed viruses were frequently detected among Black MSM in this study, including seroconverters. Further studies are needed to understand factors driving transmission and selection of CXCR4– and dual-tropic viruses among Black MSM.

Keywords

HIV; tropism; Black; men who have sex with men; dual/mixed; CXCR4; dual-tropic

INTRODUCTION

HIV entry into cells is mediated by interactions between the viral envelope (Env), CD4 receptor, and CCR5 or CXCR4 coreceptors. HIV tropism is determined by coreceptor usage; viruses can use CCR5 exclusively (R5), CXCR4 exclusively (X4), or both coreceptors (dual-tropic). Viral populations can be comprised of solely R5 or X4 viruses or include dual/mixed (DM) viruses. Individuals with X4/DM viruses often exhibit accelerated disease progression.^{1,2} While R5 viruses usually predominate early in infection, X4/DM viruses have been documented in up to 20% of recently-infected individuals.^{1,3–5} The predominance of R5 viruses during primary HIV infection is thought to result from selective advantages that favor transmission and/or replication of R5 strains.⁶ The prevalence of X4/DM viruses in recent infection is higher among people who inject drugs than among those with sexually-acquired HIV infection.^{2,3,7} This suggests that the absence of a mucosal barrier may facilitate transmission of X4/DM strains. Sexual practices (e.g., insertive vs. receptive intercourse among men who have sex with men [MSM]) may also differentially impact transmission of X4/DM viruses. These issues are relevant to HIV prevention efforts, since the CCR5 coreceptor antagonist, maraviroc, is being evaluated for use in pre-exposure prophylaxis (PrEP).^{8,9}

In this report, we explored whether sexual practices were associated with coreceptor tropism among HIV-infected MSM in the United States (US) who were enrolled in a clinical study (HIV Prevention Trials Network [HPTN] 061; NCT 00951249). This study assessed the feasibility and acceptability of a multi-component intervention to reduce HIV incidence among Black MSM who were at a high risk of HIV acquisition or transmission.^{10,11} Annual HIV incidence in the cohort was high (3.0% overall, 95% confidence interval [CI]: 2.0–4.4%; 5.9% among men 30 years old, 95% CI: 3.6–9.1%).¹⁰ We analyzed HIV tropism for the subset of study participants in HPTN 061 who were HIV infected at enrollment and reported either exclusive insertive intercourse or exclusive receptive intercourse, and among men who seroconverted during the study.

METHODS

Study cohort

HPTN 061 enrolled 1,553 Black MSM in six US cities (Atlanta, Boston, Los Angeles, New York City, San Francisco, and Washington, DC; enrollment period: 2009–2010).^{10,11} Briefly, men were recruited from the community or were referred as sexual network partners. Participants were eligible for the study if they reported at least one instance of unprotected anal intercourse with a man in the prior six months. Both HIV-uninfected and HIV-infected men were enrolled; the number of HIV-infected men in HIV care was capped at 10 men per study site. HIV testing was performed at enrollment for all participants and at study visits 6 and 12 months after enrollment for men who were uninfected at enrollment. CD4 cell count and HIV viral load were measured for men with HIV infection. All participants were tested for sexually transmitted infections. Additional testing was performed retrospectively by the HPTN Laboratory Center (Johns Hopkins University, Baltimore, MD). Participants also completed behavioral assessments via audio computer-assisted self-interview (ACASI) and answered social and sexual network questionnaires with an interviewer at each visit. The

ACASI asked participants to provide detailed information on sexual practices. Insertive intercourse was defined as insertive vaginal or anal intercourse with female, male, or transgender female/male partners; receptive intercourse was defined as receptive anal intercourse with male or transgender male partners.

Analysis of HIV tropism

HIV tropism testing was performed retrospectively using samples from two groups of men: (1) men who were HIV infected at enrollment and reported either exclusive insertive intercourse or exclusive receptive intercourse in the previous six months, and (2) men who seroconverted during the study. All samples tested had a viral load $\geq 1,000$ copies/mL. Tropism testing was performed using the Trofile assay (Monogram Biosciences, San Francisco, CA). Trofile is a recombinant phenotypic assay for determining HIV coreceptor usage.^{12,13} Briefly, full-length *env* sequences from each sample are amplified and cloned into expression vectors. Pseudoviruses are prepared by cotransfecting human embryonic kidney cell cultures with the *env* expression vectors and a replication-defective HIV genomic vector containing a luciferase reporter gene. HIV coreceptor tropism is determined by infecting CCR5⁺ and CXCR4⁺ cells in the presence and absence of CCR5 and CXCR4 inhibitors. Viral replication is quantified by measuring luciferase activity (reported as relative light units, which reflect the level of luciferase activity).¹³

Analysis of individual viral variants (clonal analysis) was performed using samples from seroconverters who had DM viruses. HIV tropism was determined for 16–20 *env* clones from the viral populations of each individual using Trofile, and the V3 loop of each clone was sequenced using standard dideoxy-chain termination.¹⁴ In addition to phenotypic tropism testing, HIV tropism was predicted using an algorithm based on V3 region sequences (the geno2pheno_[coreceptor] algorithm, version 2.5).¹⁵ The European guidelines for HIV-1 tropism testing recommend using a false positive rate (FPR) of $\leq 10\%$ to predict X4 tropism.¹⁶

Statistical methods

Correlates of HIV tropism were analyzed using Fisher's exact and chi-square tests for univariate analyses. SAS, version 9.2 (SAS Institute, Cary, NC) was used for these analyses. P values <0.05 were considered to be statistically significant.

Ethical considerations

Written informed consent was obtained from all participants in the HPTN 061 study. The institutional review boards at each participating institution approved the study.

RESULTS

In HPTN 061, 147 (42%) of the 348 men who were HIV-infected at enrollment had a viral load $\geq 1,000$ copies/mL. HIV tropism was analyzed in samples from 51 of those men: 32 (63%) reported exclusive insertive intercourse and 19 (37%) reported exclusive receptive anal intercourse (see Methods). The remaining 96 men either did not disclose their sexual practices or reported both insertive and receptive intercourse; these men were not included in

the analysis. Samples from the first HIV-positive visit were also analyzed for 21 (75%) of the 28 men who seroconverted during HPTN 061; the remaining seven men included six who had viral loads <1,000 copies/mL at the first HIV-positive visit¹⁷ and one who had no sample available for testing. HIV tropism was determined for 63 of the 72 men (Table 1): 43 who were HIV infected at enrollment and 20 seroconverters. The 43 men who were HIV infected at enrollment had a median age of 41 years (range: 18–61), a median CD4 cell count of 354 cells/mm³ (range: 20–1,849), and a median viral load of 22,551 copies/mL (range: 1,716–625,171). The 20 seroconverters had a median age of 22 years (range: 18–48), a median CD4 cell count of 563 cells/mm³ (range: 151–771), and a median viral load of 85,185 copies/mL (range: 5,933–1,732,182). All 63 men included in this study were infected with HIV-1 subtype B.¹⁸ HIV tropism was not determined for nine samples due to assay failure, including samples from five men who reported exclusive insertive intercourse, three who reported exclusive receptive intercourse, and one seroconverter.

DM viruses were detected in 11 (26%) of 43 men who were HIV infected at enrollment, including eight (30%) of 27 men who reported exclusive insertive intercourse and three (19%) of 16 men who reported exclusive receptive intercourse ($P=0.49$; data not shown). DM viruses were also detected in 5 (25%) of 20 seroconverters. One of the 20 seroconverters reported exclusive insertive intercourse and four reported exclusive receptive intercourse; the remaining 15 seroconverters reported both insertive and receptive intercourse, or did not report their sexual practices. These data were too limited to assess associations between HIV tropism and sexual practices among the seroconverters.

Since the proportion of men with DM viruses was similar among the HIV-infected men at enrollment and the seroconverters, these groups were combined to analyze factors associated with detection of DM viruses. Overall, men who had DM viruses had lower median CD4 cell counts compared to men who had R5 viruses (184 cells/mm³ vs. 386 cells/mm³; $P=0.019$; Table 1). All five seroconverters who had DM viruses had CD4 cell counts <350 cells/mm³ (median: 254 cells/mm³, range: 151–335, Table 2); in contrast, the median CD4 cell count for the seroconverters with R5 viruses was 604 cells/mm³ (range: 281–771). None of the other factors evaluated was associated with detection of DM viruses in this study.

We analyzed *env* clones from the five seroconverters who had DM viruses to determine whether their viral populations were comprised solely of dual-tropic viruses or mixtures of R5, X4, and dual-tropic viruses (Table 2). Dual-tropic viruses were further classified as either dual-R or dual-X in these analyses.^{14,19} Dual-R viruses infect CXCR4⁺ cells poorly and have *env* V3 loop sequences similar or identical to R5 viruses from the same individual, while dual-X viruses infect CXCR4⁺ cells efficiently and have *env* V3 loop sequences distinct from R5 viruses from the same individual. None of the five men had X4 viruses detected. Mixed populations of R5 and dual-R viruses were detected in two of the five men (Cases 1 and 2, Table 2); all of these viruses were predicted to be R5 viruses by the geno2pheno algorithm. There was a major subpopulation of dual-R viruses in Case 1, which had V3 sequences identical to those in one R5 clone. In Case 2, there were two proportionate subpopulations of R5 and dual-R viruses, which had identical V3 sequences. Dual-X viruses were detected in the remaining three men (Cases 3–5); all of the dual-X viruses were predicted to be X4 viruses by the geno2pheno algorithm. All clones from Cases

3 and 4 were dual-X and had identical (Case 3) or nearly identical (Case 4) V3 sequences. The viral population in Case 5 included a major subpopulation of R5 and minor subpopulations of dual-X viruses; one R5 clone was predicted to be a X4 virus by the geno2pheno algorithm.

DISCUSSION

We analyzed HIV-infected men enrolled in HPTN 061 to explore whether sexual practices were associated with HIV tropism. We did not observe a significant difference in HIV tropism among men who reported exclusive insertive vs. receptive intercourse at study enrollment. We note that the sexual practices of the men included in this analysis may have been different at the time of HIV infection than those reported for the six months preceding enrollment; self-report of sexual practices may also be unreliable in some cases. In addition, HIV evolution and superinfection could also impact the tropism of the viral population over time. Overall, DM viruses were detected among 26% of the HIV-infected men at enrollment, which is consistent with other studies that found X4/DM viruses in 20% of individuals with chronic HIV infection.¹

Twenty-five percent of the seroconverters in HPTN 061 also had DM viruses. In other studies, X4/DM viruses have been observed in 2–20% of individuals with recent HIV infection.^{1,3–5} It is difficult to compare the proportion of individuals with X4/DM viruses in different studies, since the sensitivity for detecting X4/DM viruses depends on the method used for tropism testing. In this study, we used a clinically-validated, phenotypic tropism assay with enhanced sensitivity (Trofile), which can detect X4/DM variants present in 0.3% of the viral population.¹³ Most genotypic tropism assays use bulk (population) Sanger sequencing; these methods typically detect minority variants present at 15–20%.²⁰ The sensitivity and specificity of genotypic tropism assays varies depending on the algorithms and cut-offs used to analyze V3 loop sequences.²⁰ Determinants outside the V3 loop may also impact HIV tropism.^{14,21} Those regions are included in the recombinant viruses analyzed in the Trofile assay but not in genotypic tropism assays. In two of the five seroconverters in this study, dual-tropic *env* clones were identified that had V3 loop sequences identical to those in R5-tropic clones from the same samples. Notably, HIV tropism was misclassified in these cases using a genotypic assay based on *env* V3 sequencing (the geno2pheno algorithm).

In HPTN 061, men with DM viruses had significantly lower CD4 cell counts, consistent with findings from other studies.¹ The five seroconverters with DM viruses also had low CD4 cell counts (median: 254 cells/mm³, range: 151–335). Other studies did not find a significant difference in CD4 cell count between individuals with X4/DM vs. R5 viruses early in infection,^{2,7,22} although those with X4/DM viruses had more pronounced CD4 cell count decline over time in two of those studies.^{2,7} In another study, a lower mean CD4 cell count was observed among seroconverters with DM vs. R5 viruses (450 vs. 629 cells/mm³).⁵ Low CD4 cell counts after seroconversion have been associated with faster HIV disease progression, independent of HIV tropism.^{23,24} In addition, individuals starting antiretroviral treatment (ART) with CD4 cell counts <500 cells/mm³ may not achieve the same degree of CD4 cell count recovery as those starting ART at higher CD4⁺ cell counts.²⁵

The CCR5 coreceptor antagonist, maraviroc, is currently approved for ART in the US,²⁶ and clinical trials are exploring its use for PrEP.^{8,9} Coreceptor tropism testing is recommended before using maraviroc for ART, since maraviroc may select for X4 and dual-tropic viruses.²⁷ In HPTN 061, the seroconverters with DM viruses had viral populations that included either dual-tropic viruses or mixtures of R5 and dual-tropic viruses; the efficiency of CXCR4 use varied among the dual-tropic clones from each individual. Several studies suggest that maraviroc can inhibit dual-R viruses *in vitro*.^{28–30} Two of the five seroconverters who had DM viruses in our study had mixtures of R5 and dual-R viruses, which may have been susceptible to maraviroc. However, the remaining three seroconverters had dual-X viruses, which may not be inhibited by maraviroc. Further studies are needed to determine the susceptibility of dual-tropic viruses to maraviroc. In addition, maraviroc has been shown to rapidly select for minority dual-X populations.²⁸ HIV drug resistance remains a concern for individuals who become HIV infected while taking antiretroviral drugs for PrEP. In this study, both R5 and dual-X viruses were detected in one man. Exposure to maraviroc in individuals with this tropism pattern could lead to selection of dual-X viruses, which could impact disease progression.

Black MSM in the US are disproportionately affected by HIV. Infrequent HIV testing prior to enrollment and late HIV diagnosis were common in the HPTN 061 cohort.³¹ X4/DM viruses have been associated with increases in viral load, more rapid CD4 cell decline, and faster disease progression.^{1,2} The high prevalence of X4/DM viruses among men in HPTN 061 highlights an urgent need to increase HIV testing frequency in this population. Further research is also needed to identify factors driving transmission and selection of X4/DM viruses and to evaluate potential associations of HIV tropism with disease progression and treatment outcomes among Black MSM.

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Table 1

Association of HIV tropism with demographic, clinical, and behavioral factors.

Characteristic	Total 63	DM 16	R5 47	P value
City				
Atlanta	12 (19%)	3 (19%)	9 (19%)	0.26
Boston	5 (8%)	1 (6%)	4 (9%)	
Los Angeles	24 (38%)	7 (44%)	17 (36%)	
New York City	9 (14%)	1 (6%)	8 (17%)	
San Francisco	7 (11%)	4 (25%)	3 (6%)	
Washington, DC	6 (10%)	0 (0%)	6 (13%)	
Age				
30 years	27 (43%)	7 (44%)	20 (43%)	0.93
>30 years	36 (57%)	9 (56%)	27 (57%)	
Sexual identity				
Homosexual/gay	30 (48%)	9 (56%)	21 (45%)	0.76
Bisexual	16 (25%)	3 (19%)	13 (28%)	
Other	17 (27%)	4 (25%)	13 (28%)	
Education				
High school or less	34 (54%)	9 (56%)	25 (53%)	0.83
At least some college	29 (46%)	7 (44%)	22 (47%)	
Household income				
\$30,000/year	51 (81%)	12 (75%)	39 (83%)	0.48
>\$30,000/year	12 (19%)	4 (25%)	8 (17%)	
Employment status				
Employed	15 (24%)	5 (31%)	10 (21%)	0.50
Unemployed	48 (76%)	11 (69%)	37 (79%)	
Student status				
Student	18 (29%)	5 (31%)	13 (28%)	0.76
Non-student	45 (71%)	11 (69%)	34 (72%)	
Circumcision status				
Circumcised	51 (81%)	13 (81%)	38 (81%)	1.00
Uncircumcised	12 (19%)	3 (19%)	9 (19%)	
STI at enrollment				
Yes	13 (21%)	2 (13%)	11 (23%)	0.49
No	50 (79%)	14 (88%)	36 (77%)	
Median viral load (copies/mL)	22,551	56,152	20,236	0.28
Median CD4 count (cells/mm ³)	357	184	386	0.019
In prior six months:				
Number of male partners				
0–1	13 (21%)	5 (31%)	8 (17%)	0.29
>1	50 (79%)	11 (69%)	39 (83%)	

Characteristic	Total 63	DM 16	R5 47	P value
Gender of partners				
Male only	42 (67%)	9 (56%)	33 (70%)	0.31
Male and female	21 (33%)	7 (44%)	14 (30%)	
Had a new male partner				
Yes	51 (81%)	11 (69%)	40 (85%)	0.16
No	12 (19%)	5 (31%)	7 (15%)	
Race/ethnicity of partners				
All Black	41 (65%)	10 (63%)	31 (66%)	0.89
Some Black	18 (29%)	5 (31%)	13 (28%)	
None Black	4 (6%)	1 (6%)	3 (6%)	
Unprotected receptive anal intercourse				
Yes	31 (49%)	6 (38%)	25 (53%)	0.28
No	32 (51%)	10 (63%)	22 (47%)	
Unprotected insertive anal intercourse				
Yes	30 (48%)	8 (50%)	22 (47%)	0.83
No	33 (52%)	8 (50%)	25 (53%)	
Received money/goods for sex				
Yes	13 (21%)	3 (19%)	10 (21%)	1.00
No	50 (79%)	13 (81%)	37 (79%)	
Provided money/goods for sex				
Yes	7 (11%)	1 (6%)	6 (13%)	0.67
No	56 (89%)	15 (94%)	41 (87%)	
Alcohol problem ^a				
Yes	14 (22%)	4 (25%)	10 (21%)	0.74
No	49 (78%)	12 (75%)	37 (79%)	
Substance use ^b				
Yes	41 (65%)	11 (69%)	30 (64%)	0.72
No	22 (35%)	5 (31%)	17 (36%)	

The table shows associations between HIV tropism and demographic, clinical, and behavioral factors for all 63 men with HIV tropism data, including 43 men who were HIV-infected at enrollment and 20 HIV seroconverters. P values <0.05 are bolded. Collection of demographic and behavioral data has been previously described in detail.^{10,11} Abbreviations: DM: dual/mixed viruses; R5: CCR5-using viruses; STI, sexually transmitted infection.

^aAlcohol use was defined as having a score ≥ 8 using the Alcohol Use Disorders Identification Test (AUDIT).

^bSubstance use included inhaled nitrates, smoked and powder cocaine, methamphetamine, heroin, nonprescription drug use (Oxycontin, Vicodin, or Xanax), or any other hallucinogens. Injection drug use was reported in only four (6%) of 63 men included in this analysis. This included three men who were HIV infected at enrollment (two with R5 viruses and one with DM viruses) and one seroconverter (with R5 viruses).

Table 2Coreceptor usage and V3 loop sequences of *env* clones from HIV seroconverters with dual/mixed viruses.

Case No.	Viral Load ^a	CD4 Count ^b	No. of Clones ^c	Tropism (Trofile) ^d	CCR5 ⁺ cell infectivity (RLU) ^e	CXCR4 ⁺ cell infectivity (RLU) ^e	FPR (%) ^f	V3 loop amino acid sequences
1	242,022	334	9	Dual-R	57,874-381,187	1,160-28,202	94.1	CTRPNNNTRK <u>S</u> IHIAPEGRAFFATGDIIGDIRQAHC
			2	R5	53,141-91,918	57-72	86.5	N.....Y....M.....
			2	Dual-R	92,523-179,041	8,182-15,549	83.0	Y.....
			1	R5	57,634	69	92.2	N.....Y.....
			1	R5	56,344	65	83.0	Y.....
			1	R5	20,159	71	94.1	
			1	Dual-R	202,966	1,525	74.6	.A.....
			1	Dual-R	191,955	11,286	79.7	V.....
			1	Dual-R	153,724	3,106	86.8	K..Y.....
2	141,411	254	8	Dual-R	50,401-229,518	302-2,472	38.4	CTRPGNNTRK <u>S</u> IHIGPGRFYATGDIIGDIRQAHC
			6	R5	33,694-131,674	50-103	38.4	
			1	R5	38,299	79	14.3	.A.....
			1	Dual-R	229,518	2,014	34.6	M.....
3	90,920	335	18	Dual-X	494-211,474	96,584-1,451,417	0.5	CTRPNNNTRK <u>R</u> MSLGPGKVFTTGGIIGDIRKAHC
4	165,010	235	10	Dual-X	1,804-19,776	16,153-87,607	2.1	CTRPNNNTRK <u>S</u> IRIGPGRWSVFATGKIIGDIRQAHC
			3	Dual-X	1,783-4,628	15,345-35,774	1.1	E.....R...
			2	Dual-X	3,011-31,528	38,768-128,306	1.7	R...
			1	Dual-X	2,163	16,759	1.7	T.....
5	79,450	151	11	R5	30,379-154,907	55-99	50.9	CSRPNNNTRK <u>S</u> ISIGPGRFYATGDIIGDIRQAHC
			2	Dual-X	49,924-65,980	396,970-629,351	3.9	G..R.....T.....R.....
			2	Dual-X	8,888-19,161	86,732-185,976	4.7	R.....T.....R.....
			1	R5	58,306	73	6.0	G.....

The table shows results of *env* clonal analyses for five HIV seroconverters with DM viruses. Amino acid positions 11 and 25 in the *env* V3 loop are underlined. Abbreviations: no.: number; RLU: relative light units of luciferase output from the Trofile assay; FPR: false-positive rate for geno2pheno[coreceptor].

^aViral load was measured as copies/mL.

^bCD4 cell count was measured as cells/mm³.

^cThe total number of clones in each individual with the indicated V3 loop sequence.

^dHIV tropism for each *env* clone was determined using the Trofile Assay. Clones were identified as CCR5-using (R5), CXCR4-using (X4), or dual-tropic virus. Dual-tropic viruses were further classified as dual-R or dual-X according to their CXCR4⁺ cell infectivity and V3 loop sequence.

^eThe minimum to maximum RLU is shown in cases with multiple distinct clones.

^fA false positive rate (FPR) of 10% was used to predict X4 tropism using an algorithm based on V3 region sequences (geno2pheno[coreceptor]). A FPR = 10% predicts X4 tropism; a FPR >10% predicts R5 tropism.