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Determining HIV-1 Coreceptor Tropism Using PBMC Proviral DNA Derived from Aviremic Blood Samples

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BACKGROUND

- HIV-1 utilizes CD4 and either the CCR5 and/or CXCR4 co-receptor to enter the host cell.
- Agents that block the ability of HIV to utilize CCR5 are available for use in the clinic or in late stage development.
- Determining the tropism of a patient's virus prior to CCR5 antagonist therapy is recommended.
- Current tropism assays are routinely performed using envelope (*env*) sequences from plasma viral RNA and thus cannot be used to evaluate patients with undetectable levels of plasma virus.
- To address this limitation, we have begun to adapt a pseudovirion-based tropism assay to utilize PBMC-derived *env* sequences.

METHODS

- Plasma and PBMC samples were obtained from 22 patients in the UCSF SCOPE cohort. CD4 counts, HIV VL and nadir CD4 values are shown for each patient.
- Coreceptor tropism of plasma samples was determined using the Trofile assay.
- Relative ability to infect U87 cells expressing CD4 and CCR5 or CXCR4 is shown for viral pools and clones. The magnitude of infectivity is based on luciferase production in the U87 target cells
- Genomic DNA was extracted from PBMC samples and amplified gp160 *env* sequences were processed through the Trofile assay.
- Multiple *env* clones were isolated from PBMC-associated DNA from 5/12 of the aviremic samples. Tropism and gp160 sequences of *env* clones were determined.
- Additionally, multiple *env* clones were isolated from four samples with paired plasma and PBMC virus populations and the tropism and gp160 sequences of those *env* clones were determined.
- gp160 sequences were aligned and NJ phylogenetic trees were constructed with HXB2 sequences as the root.
- Phenotypically determined tropism results from *env* clones were compared to predictions of tropism based on clone V3 sequences (SVM, PSSM and 11/25 rule).

Table 1: Tropism determination utilizing *env* amplified from PBMC-derived DNA in twelve aviremic HIV+ patients

Sample ID	Tropism	Magnitude of Infectivity R5	Magnitude of Infectivity X4	CD4	VL	Nadir CD4
PBMC_1	R5	+++	-	711	43	10
PBMC_2	R5	++	-	930	<40	118
PBMC_3	R5	+++	-	420	<40	268
PBMC_4	R5	++	-	383	<40	98
PBMC_5	R5	+++	-	606	<40	187
PBMC_6	R5	++	-	274	<40	27
PBMC_7	DM	+++	++	289	<40	133
PBMC_8	DM	++	++	126	<40	37
PBMC_9	DM	++	+++	264	<40	50
PBMC_10	DM	+++	++	290	<40	24
PBMC_11	DM	+++	+++	203	<40	19
PBMC_12	X4	-	+++	276	<40	38

Table 2: V3 sequence and tropism diversity among *env* clones amplified from PBMC-derived DNA

Sample ID	No. of clones	V3 sequences	Tropism	Average Magnitude of Infectivity R5	Average Magnitude of Infectivity X4	V3 Tropism Prediction SVM	V3 Tropism Prediction PSSM	V3 Tropism Prediction 11/25
PBMC_1	9	CTRPNNNTRKGVHIGPGSVWYTTGEIIG-DIRQAHC	R5	+++	-	R5	R5	R5
PBMC_2	16	CTRPNNNTRRGIHIGPGRAFYTTGEITG-DIRQAHC	R5	++	-	R5	R5	R5
PBMC_8	5	CTRPGNNTRRSITIGPGRAFYTN-NIIG-DIRQAYC	Dual	++	+	X4	R5	R5
	3	.....-.....-N.....	Dual	++	+	X4	R5	R5
	2	....S.K..K...R....VY..T--.G.KS.K..F.	X4	-	++	X4	X4	R5
PBMC_9	8	CTRPHNTIRRRIHIGPGRAFYT-GQTTGDIRKAHC	Dual	++	+++	X4	X4	X4
	4	....N.NT..G.....T..I.....	Dual	++	+	R5	R5	R5
	1	.....V.....T..I.....	Dual	+	++	X4	X4	X4
	1	.....-.....	R5	+	-	X4	X4	X4
PBMC_12	14	CTRPSNKTRKKVTLGPGKVWYTTEVKGDIRKAHC	X4	-	++	X4	X4	X4
	1	.....V..	X4	-	++	X4	X4	X4

Figure 1: Diversity of gp160 sequences and tropism in *env* clones derived from PBMC-associated DNA

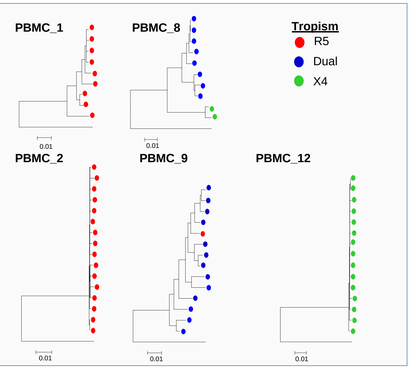


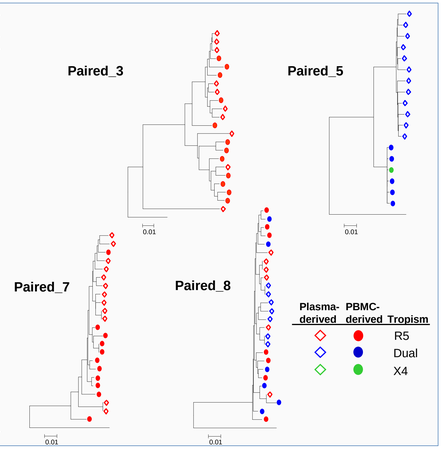
Table 3: Concordant tropism results in *env* sequences amplified from plasma RNA and PBMC-associated DNA in ten viremic patients

Sample ID	Source	Tropism	Magnitude of Infectivity R5	Magnitude of Infectivity X4	CD4	VL	Nadir CD4
Paired_1	Plasma	R5	++	-	1048	15,100	877
	PBMC	R5	++	-			
Paired_2	Plasma	R5	+++	-	821	19,251	589
	PBMC	R5	++	-			
Paired_3	Plasma	R5	++	-	453	313,000	343
	PBMC	R5	++	-			
Paired_4	Plasma	R5	++	-	449	90,814	449
	PBMC	R5	++	-			
Paired_5	Plasma	DM	++	++	40	53,600	26
	PBMC	DM	++	+++			
Paired_6	Plasma	R5	++	-	nd	78,000	nd
	PBMC	R5	+++	-			
Paired_7	Plasma	R5	+++	-	431	23,700	272
	PBMC	R5	+++	-			
Paired_8	Plasma	R5	++	-	429	22,275	393
	PBMC	R5	++	-			
Paired_9	Plasma	DM	+	++	11	840	3
	PBMC	DM	+++	++			
Paired_10	Plasma	R5	+++	-	453	505,000	343
	PBMC	R5	+++	-			

Table 4: V3 sequence and tropism diversity among *env* clones amplified from plasma RNA and PBMC-derived DNA in four viremic patients

Sample ID	No. of clones	V3 sequences	Tropism	Ave Magnitude of Infectivity R5	Ave Magnitude of Infectivity X4	V3 Tropism Prediction SVM	V3 Tropism Prediction PSSM	V3 Tropism Prediction 11/25	No. Clones Plasma	No. Clones PBMC
Paired_3	9	CARPNNNTRKSIHIGPGKAFYTTGSIIGDIRQAHC	R5	+++	-	R5	R5	R5	6	3
	1	.....S....F.....	R5	++	-	R5	R5	R5	1	0
	1	.T..G.....R.M.A..Q.....	R5	+++	-	R5	R5	R5	1	0
	1	.T.....A..RS..A..A.....	R5	+++	-	R5	R5	R5	1	0
	8	.T.....A..RS..A..D...N.....	R5	+++	-	R5	R5	R5	1	7
	1	.....F.....	R5	+++	-	R5	R5	R5	0	1
	1	.V.....	R5	+++	-	R5	R5	R5	0	1
Paired_5	11	CTRPNNNTKKGITMGPGRVYYTTGQIVGDIRRAHC	Dual	+++	+++	R5	X4	R5	11	0
	2	.....Y.	Dual	+++	+++	X4	X4	R5	2	0
	4	.....K.I.....Y.	Dual	++	+++	X4	X4	X4	0	4
	1	....D.....K.I.....Y.	Dual	++	++	X4	X4	X4	0	1
	1	.....R.....K.I.....Y.	X4	-	++	X4	X4	X4	0	1
Paired_7	15	CTRPNNNTRKGIHIGPGSAFYATGEVIGDIRQAYC	R5	+++	-	R5	R5	R5	9	6
	2	....G.....S.....AII...K..F.	R5	+++	-	R5	R5	R5	2	0
	1	.....N.....	R5	+++	-	R5	R5	R5	1	0
	2	....G.....	R5	+++	-	R5	R5	R5	0	2
	1	....A.....	R5	+++	-	R5	R5	R5	0	1
	1	....G.....II.....H.	R5	+++	-	R5	R5	R5	0	1
	1	.....AII.....	R5	+	-	R5	R5	R5	0	1
Paired_8	7	CTRPSNNTRKSIHIGFGRALYATGRIIGDIRQAHC	R5/Dual	++	+	R5	R5	X4	6	1
	4	.....	R5	++	-	R5	R5	X4	3	1
	2	.....A.....	Dual	+++	+	R5	R5	X4	2	0
	1	.....G.....K..	R5	++	-	R5	R5	X4	1	0
	3	.....G.....K..	R5	++	-	R5	R5	X4	1	2
	2	.....N.....	R5/Dual	+++	+	R5	R5	X4	0	2
	2	.....V.....	R5/Dual	++	+	R5	R5	X4	0	2
	1	.....N.....E....M.....	Dual	+++	+	R5	R5	R5	0	1
	1	.....N.....K.....	Dual	++	+	R5	R5	X4	0	1
	1	.....V....K..	Dual	++	+	R5	R5	X4	0	1
	1	.....N.....E.....	R5	++	-	R5	R5	R5	0	1
	1	.....G.....	R5	+++	-	R5	R5	X4	0	1

Figure 2: Diversity of gp160 sequences and tropism in *env* clones derived from paired plasma RNA and PBMC-associated DNA



RESULTS

- Tropism was successfully determined in all 22 PBMC samples and in 10/10 viremic plasma samples. The remaining 12 plasma samples had low or undetectable viral loads.
- Of the 12 aviremic patients, six PBMC proviral DNA-derived viruses were R5, five were DM and one was X4 (Table 1).
- Clonal analysis of PBMC-derived *env* in 5/12 aviremic samples shows two samples made up of relatively homogenous R5 clones, one sample entirely composed of closely related X4 clones and two samples with majority dual-tropic clones (Table 2, Figure 1).
- In the 10 viremic patients, PBMC and plasma tropism determinations were concordant (8 R5, 2 DM) (Table 3).
- *env* sequences derived from plasma and PBMC from the same patient exhibited extensive homology. Clonal analysis did not reveal phylogenetic distinctions between plasma and PBMC samples from 3/4 paired samples (Table 4, Figure 2).
- Tropism of clones derived from plasma and PBMC were concordant (Figure 2): one DM virus population was comprised of dual-tropic variants with one X4 clone; one R5 virus population was comprised predominantly of R5 variants with a minor subpopulation of dual-tropic variants which weakly utilized CXCR4 for infection; the two R5 virus populations were comprised of R5 variants.

CONCLUSIONS

- Full-length *env* can be efficiently amplified from cell-associated HIV-1 DNA of patient PBMC to determine coreceptor usage.
- Viral tropism and phylogenetic relatedness of virus populations and clones were generally concordant between paired plasma and PBMC samples.
- The ability to test tropism using cell-associated HIV may be applicable to determining the suitability of CCR5 antagonist use in patients with low or undetectable plasma viral load.

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