Additionally, multiple Genomic DNA was extracted from PBMC samples and To address this limitation, we have begun to adapt a Determining the tropism of a patient’s virus prior to CCR5 HIV-1 utilizes CD4 and either the CCR5 and/or CXCR4 co-

METHODS

cells with paired plasma and PBMC virus populations env DNA from 5/12 of the aviremic samples. Tropism and gp160 magnitude of infectivity is based on luciferase production in or CXCR4 is shown for viral pools and clones. The Trofile assay.

in the UCSF SCOPE cohort. CD4 counts, HIV VL and nadir receptor to enter the host cell.

plasma RNA and PBMC-associated DNA tropism in

Figure 1: Diversity of gp160 sequences and Figure 2: Diversity of gp160 sequences and tropism in en clones derived from plasma RNA and PBMC-associated DNA TABLE 3: Concordant tropism results in env sequences amplified from plasma RNA and TABLE 4: V3 sequence and tropism diversity among env clones derived from plasma RNA and PBMC-associated DNA in four viremic patients

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

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

Table 3: Concordant tropism results in env sequences amplified from plasma RNA and TABLE 4: V3 sequence and tropism diversity among env clones derived from plasma RNA and PBMC-associated DNA in four viremic patients

RESULTS

• Tropism was successfully determined in all 22 PBMC samples and in 10/15 viremic plasma samples. The remaining 12 plasma samples had high 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 env in 5/12 aviremic samples shows two samples made up of relatively homogenous RS 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, plasma and PBMC tropism determinations were concordant (R 5, DM 2) (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 8/15 viremic samples (Table 4, Figure 2).

• Tropism of clones derived from plasma and PBMC were concordant (Figure 2): one DM HIV virus population was comprised of dual-tropic variants with one X4 clone and one X4 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 co-receptor usage.

• Viral tropism and phylogenetic relationship 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.

ACKNOWLEDGEMENTS

• We are grateful to the Monogram Biosciences Clinical Reference Lab for their assistance in performing Trofile assays used in this study.

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Table 4: V3 sequence and tropism diversity among env clones derived from paired plasma RNA and PBMC-associated DNA

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<th>Sample ID</th>
<th>Tropism</th>
<th>Magnitude of Infectivity</th>
<th>CD4</th>
<th>Sample ID</th>
<th>Tropism</th>
<th>Magnitude of Infectivity</th>
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<td>X4</td>
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<td>-</td>
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<tr>
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