

Predicted Co-receptor Usage of HIV-1 in Low-level Viremias during HAART

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Abstract

Background: While most low-level viremia (LLV) appears to be derived from the expression of archived virus, some low-level viremias are from active viral replication. We hypothesized that low-level viremia from individuals with continued viral replication during HAART would use the CXCR4 chemokine receptor, while expressed archived virus would use the CCR5 chemokine receptor, based on when in the course of disease that each of these types of viruses were selected (late vs early, respectively).

Methods: A bioinformatic method based on a position-specific scoring matrix of the V3 loop of *env* (sensitivity 84%, specificity 96%) was used to predict co-receptor use of low-level viremia sequences obtained by endpoint dilution PCR. In addition, co-receptor use from the 1 subject with non-subtype-B (subtype-D) virus was predicted based on basic amino acid residues at V3 positions 11 and 25.

Results: From 8 subjects' low-level viremia specimens, 45 sequences encoding the C2-V5 region of *env* were available for prediction of co-receptor usage; 7 of the 8 subjects also had phenotype predicted by culture in MT-2 cells prior to initiation of HAART. CXCR4 receptor use was predicted for 16 of 45 sequences. These 16 sequences came from low-level viremia of 2 subjects (2/2 sequences from 1 low-level viremia episode of 1 subject, and 14/16 from 5 low-level viremia episodes in the second subject). The remaining 27 sequences, from 6 subjects were predicted to use the CCR5 chemokine receptor. Pre-HAART MT-2 results detected syncytium-inducing (presumably X4) virus in 3 of 7 subjects including 1 of 2 subjects with CXCR4 co-receptor use in low-level viremia. The 2 subjects whose low-level viremia sequences were predicted to use the CXCR4 chemokine receptor correlated to 2 of 11 subjects predicted to have active viral replication during HAART.

Conclusions: Viral co-receptor use by low-level viremia sequences appears to correspond with the mechanism causing the low-level viremia: low-level viremia from subjects with viral replication during HAART predominately used the CXCR4 co-receptor, and low-level viremia from expression of archived virus used the CCR5 co-receptor. These data further support our hypothesis that these 2 distinct phenomena cause low-level viremia. Further studies of larger populations are warranted to determine whether low-level viremia co-receptor use can distinguish between ongoing viral replication vs expression of archived virus, due to differences in their clinical consequences.

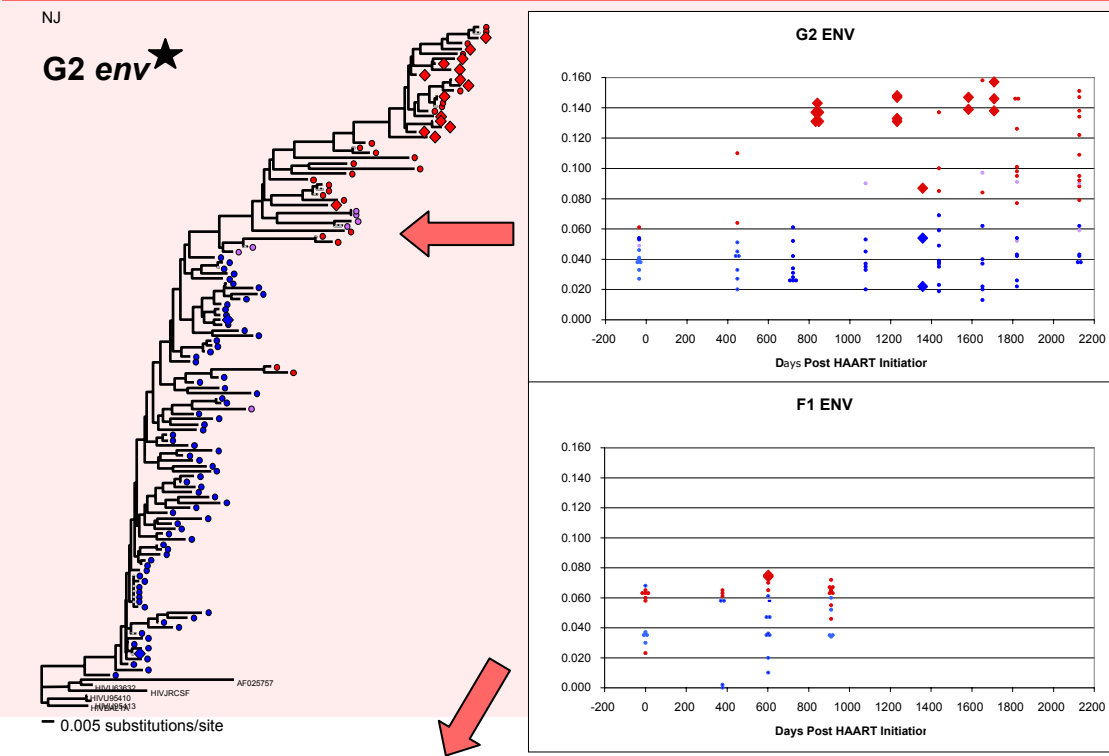
Methods

- Previously obtained sequences from plasma low-level viremias (LLV) and peripheral blood mononuclear cells (PBMC) sequences were evaluated for predicted co-receptor use.
- Plasma LLV sequences had been obtained by end-point dilution PCR after specific reverse transcription.
- PBMC sequences were obtained by end-point dilution PCR.
- Co-receptor usage in the 7 subjects with subtype B virus was evaluated using a position-specific scoring matrix of the V3 loop of *env*¹.
- Co-receptor usage in subject F1, with subtype D virus, was predicted using the 11/25 or charge method² as syncytium-inducing (SI), and presumably X4 virus, if arginine (R) was encoded at position 306 in V3 or arginine or lysine (K) were encoded at position 320 of the V3 loop of gp120.
- Divergence of plasma (low-level viremia) and PBMC sequences from the inferred most recent common ancestor of infection (MRCA) during HAART³ was examined in relation to predicted co-receptor usage.

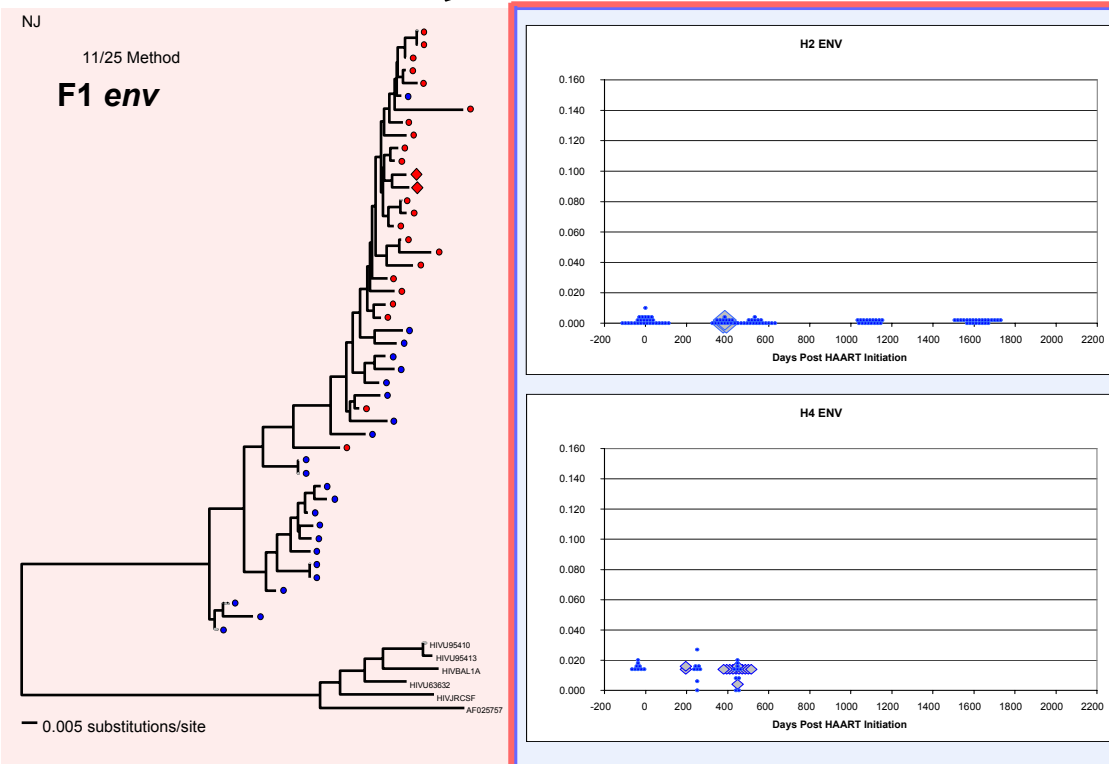
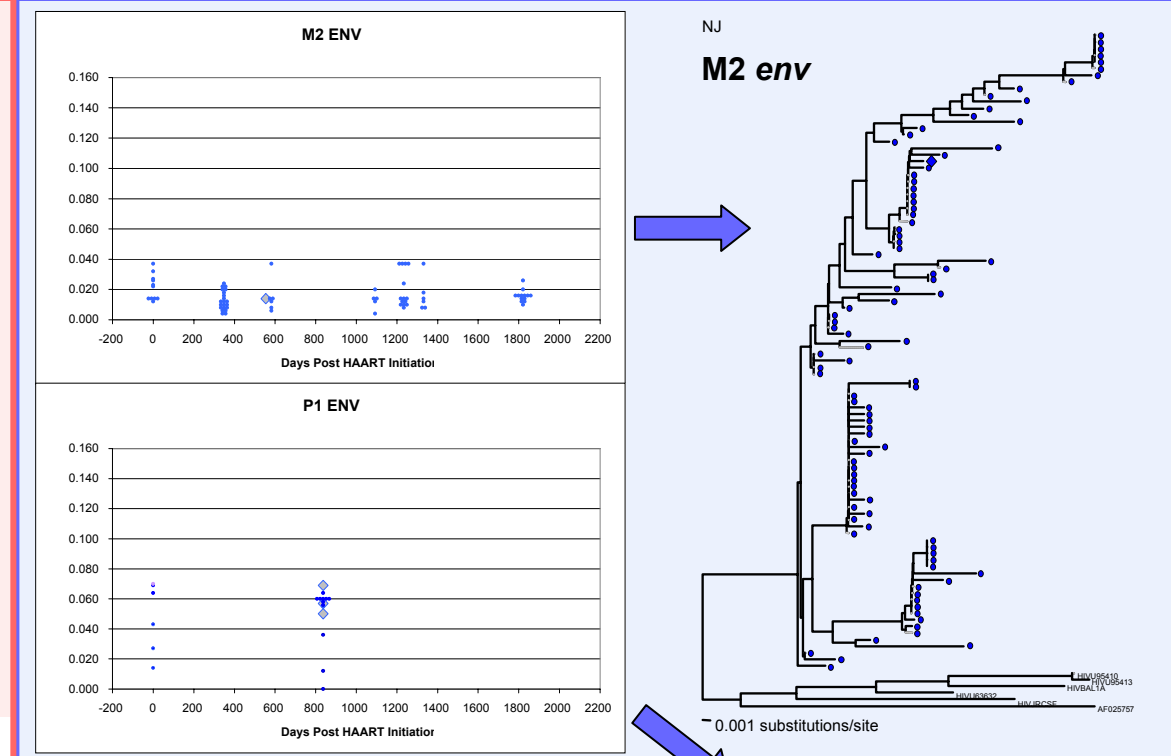
References:

- Jensen MA et al. Improved Coreceptor Usage Prediction and Genotypic Monitoring of R5-to-X4 Transition by Motif Analysis of Human Immunodeficiency Virus Type 1 *env* V3 Loop Sequences. *J Virol* 2003 77:13376-88.
- Fouchier RAM et al. Simple Determination of Human Immunodeficiency Virus Type 1 Syncytium-Inducing V3 Genotype by PCR. *J Clin Micro* 1995 33:906-911.
- Tobin NH et al. Low-level Viremias During Effective HAART Result From Two Processes. 2004 *In Review*.

2 Subjects with X4-virus in Low Level Viremia



6 Subjects with R5-virus in Low Level Viremia



Legend

- ◆ OR ◆ Plasma virus - R5
- ◆ Plasma virus - X4
- Cell virus - R5
- Cell virus - Indeterminate
- Cell virus - X4
- ★ To evaluate whether the divergence from the Most Recent Common Ancestor of Infection (MRCA) in subject G2 was due to evolution in the V3 loop of *env*, we constructed a new phylogenetic tree using the same model but excluding the V3 loop of *env*. We found that up to 25% of the divergence was attributable to the V3 loop, but the overall relationship of sequences remained unchanged (data not shown).

Syncytium Inducing (SI, presumably X4 virus) by MT-2 Culture vs. Genotype Prediction:

Subject	MT-2 Cell Culture Prior to HAART ¹		X4 by Genotype	
	SI-HIV Detected	#SI+/#MT-2 cultures	X4 in PBMC	X4 in LLV
F1	Yes	1/1	Yes	Yes
G2	No	0/8	Yes	Yes
H2	Never Tested	0/0	No	No
H4	No	0/1	No	No
M2	Yes	7/12	No	No
P1	No	0/1	Indeterminate	No
S1	Yes	1/11	No	No
V1	No	0/3	No	No

- G2 & M2 had a non-syncytium-inducing virus within the 1st year of HAART (included above)
- Syncytium-inducing (SI) was determined using supernatants from PBMC Culture.

Summary

- The 2 subjects previously found to have persistent viral replication during HAART had mostly CXCR4-tropic virus in their LLV by genotype.
- The 6 subjects without evidence of viral replication during HAART had CCR5-tropic virus in their LLV by genotype.
- Pre-HAART MT-2 culture indicated syncytium-inducing (presumably X4 virus) in 3 subjects, including one of the subjects with CXCR4-tropic virus in LLV.
- 75% of the evolution in the *env* sequences with X4 genotype of subject G2 was outside the V3 loop of gp120, excluding the V3 loop as the principal contributor of phylogenetic divergence.

Comment

- Evaluation of HIV-1 co-receptor use in episodes of LLV could potentially differentiate subjects with ongoing viral replication during HAART from those with expression of archived virus.