Role of HIV-1 Diversity in Sensitivity to Treatment with Monoclonal Antibodies

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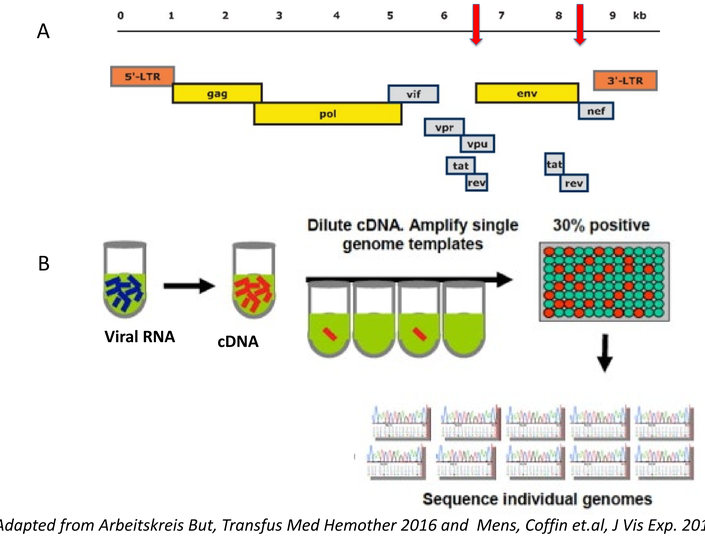
Introduction

Antiretroviral therapy (ART) can successfully suppress HIV-1 replication, however, patients on ART must maintain consistent therapy in order to avoid rebound from a latent viral reservoir. Strategies are urgently needed to clear or limit this reservoir. Individuals infected for longer periods with replicating virus prior to ART harbor greater viral genetic diversity in their reservoir. Broadly neutralizing antibodies (bNAbs) have been identified and are capable of neutralizing highly diverse viruses. More studies are needed to explore the role viral diversity plays in the potential efficacy of treatment with bNAbs. To do so, we have characterized HIV-1 genetic diversity in individuals with varying lengths of infection, as well as the sensitivity of their inducible virus reservoir to bNAbs.

Methods

Peripheral blood mononuclear cells were collected from 8 well-characterized HIV-1+ males on ART with varied lengths of active infection. Resting CD4 T cells were plated at multiple dilutions in replicate and maximally stimulated to induce latent viruses to grow in the supernatants. Cultures were screened for the presence of virus antigen and those that were positive were collected for analysis. In order to genetically define each person's viral reservoir, the HIV-1 envelope gene (*env*) was amplified and sequenced from each culture by single genome sequencing (SGS). At least 3 sequences were obtained from each culture well and 3-5 wells were sequenced from each individual. All sequences from an individual were aligned and used to generate a maximum-likelihood tree. If we confirmed a single virus was contained in the well, all well sequences were then used to generate a well consensus sequence, which were aligned to produce a maximum-likelihood tree. Finally, cultures confirmed by sequencing to contain single viruses were then titered and measured for sensitivity to bNAbs in the standard HIV-1 TZM-bl neutralization assay.

Figure 1: Amplifying *env* genes



Adapted from Arbeitskreis But, Transfus Med Hemother 2016 and Mens, Coffin et.al, J Vis Exp. 2011

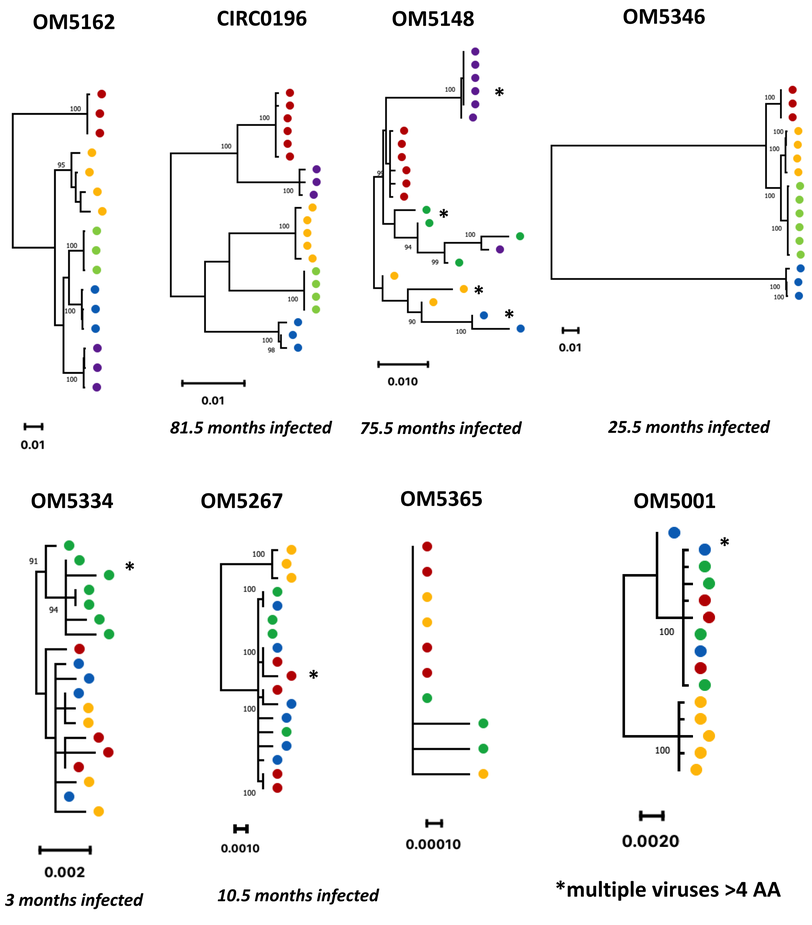
(A) Structure of the HIV-1 genome. (B) Viral RNA was extracted from each wells' supernatants to produce cDNA, using reverse genetics. The HIV-1 envelope gene (*env*) was amplified using SGS and Sanger sequencing from these reservoir-derived outgrowth viruses.

Table 1: Clinical Characteristics of Study Participants

Participant ID	Age	Sex	Viral load (copies/mL)	CD4 Count	HIV Clade	Estimated length of unsuppressed infection (months)	Duration of ART (Years)	IUPM
OM5346	48	M	N/A	1.182x10^9/L	B, AG	25.5	5	0.27
OM5148	47	M	N/A	0.733x10^9/L	B	75.5	10	1.02
OM5334	33	M	N/A	0.812x10^9/L	B	3	3	1.57
OM5365	56	M	N/A	0.624X10^9/L	B	>18	25	0.42
OM5267	29	M	N/A	0.429X10^9/L	B	10.5	3	2.34
OM5001	43	M	42	0.540x10^9/L	B	>14	9	10.46
OM5162	53	M	N/A	0.478X10^9/L	B	>3	14	0.65
CIRC0196	56	M	N/A	0.679x10^9/L	B	81.5	3	0.49

Ren *et al., Journal of Virology*, 2018

Figure 2: Viral Reservoir Diversity using Maximum Likelihood Trees



Maximum likelihood trees for each participants' sequencing. Trees are rooted at midpoint for visualization. Each dot represents a single sequence and is colored based on the well it originated from. Bootstraps over 90% are indicated. Trees revealed 80% of wells harbored a single virus. Despite utilizing a very small sample size in the analysis, we observed a clear trend toward lower diversity in individuals with short active infections.

Figure 3: Maximum likelihood tree of participant reservoir viruses

Maximum likelihood tree of gapstripped amino acid consensuses from each outgrowth virus based on trees from Figure 2. Wells containing multiple viruses were excluded from the tree. Participant OM5346 was co-infected with subtypes B and AG viruses. For individuals with higher levels of viral diversity, no wells had identical viruses and branched separately; however, low-diversity individuals harbored essentially 1-2 different viruses.

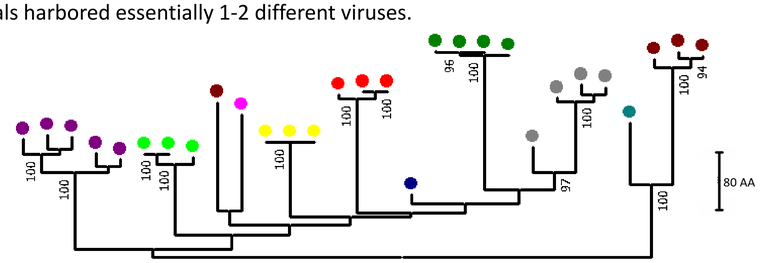
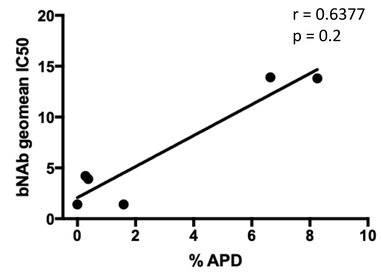


Figure 4: Participants with greater reservoir diversity display greater bNAb resistance

A

Participant	Average pairwise distance	Average Geomean IC50 for bNAbs
OM5346	19.20%	4.40
OM5334	0.28%	4.22
OM5365	0.00%	1.42
OM5267	0.37%	3.90
OM5001	1.59%	1.42
OM5162	8.26%	13.78
CIRC0196	6.64%	13.95

B



(A) Average pairwise distance (APD) was calculated as a measure of reservoir diversity for each participant in the study using DIVEIN (Deng *et al.*, *Biotechniques*, 2010).
(B) Each virus was tested for neutralization sensitivity against a panel of 10 HIV-1 bNAbs: CD4bs targeting antibodies VRC01, VRC07-523, 3BNC117, and N6, V1V2 targeting antibodies PGDM1400, CAP256.VRC26.25, and PG09, V3 targeting antibodies PGT121 and 10-1074, and MPER targeting antibody 10E8. Geometric mean IC50s were calculated against this panel as a measure of overall bNAb sensitivity for each participant. Spearman's correlations were performed to determine the relationship between APD and bNAb sensitivity. Interestingly, while these factors are not statistically significant in our data set, there is a trend towards greater resistance to bNAbs in individuals who have greater diversity. APD could not be calculated for participant OM5148 due to QVOA wells containing multiple viruses as determined by SGS.

Conclusions

- SGS revealed differences in reservoir diversity between participants, with some individuals harboring unique viruses while others harbor a few clonal strains.
- Our data suggests that average pairwise distance (APD) is associated with greater resistance to bNAbs; however, individuals who start ART early in infection and develop limited virus variation are more likely to be sensitive to bNAb treatment.

Acknowledgements

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