Characterizing Antibody Responses in ART-treated Individuals

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Introduction

Although antiretroviral therapy (ART) suppresses HIV-1 replication, ART-treated individuals must maintain therapy to avoid rebound from a viral reservoir. Strategies to limit or clear this reservoir are urgently needed, and will likely require multi-pronged approaches. Broadly neutralizing antibodies (bNAbs) could serve as one such prong, but understanding the role of autologous antibodies can help inform when bNAbs will have the greatest effect. Recent research has indicated that individuals living with HIV-1 longer prior to treatment may harbor more diverse reservoirs[1], but also that these patients often exhibit broader anti-HIV-1 antibody responses[2]. The roles that virus diversity and length of treatment play in the humoral immune response must be further studied to inform multi-pronged approaches to clearing infection. Here, we aim to elucidate roles for autologous antibodies in these treatments by characterizing the functional anti-HIV-1 antibody response in individuals with different lengths of infection pre-ART.

Methods

Plasma samples were obtained from 8 male participants on ART, and indirect ELISA assays were performed to detect binding against HIV-1 gp41 and gp120 Env proteins. Polyclonal IgG was isolated by melon gel and HIV-1 neutralization assays were performed according to the standard Tzm-bl assay to test neutralization on a global panel of HIV-1 viruses, as well as autologous and heterologous QVOA virus outgrowth supernatants. QVOA viruses were also tested against a panel of bNAbs for neutralization sensitivity. SGS was performed on outgrowth viruses, and maximum-likelihood trees of NT sequences were constructed with ≥3 sequences obtained per QVOA well.

Table 1: Clinical characteristics of study participants[3]

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

Figure 1: Maximum likelihood tree of participant reservoir viruses

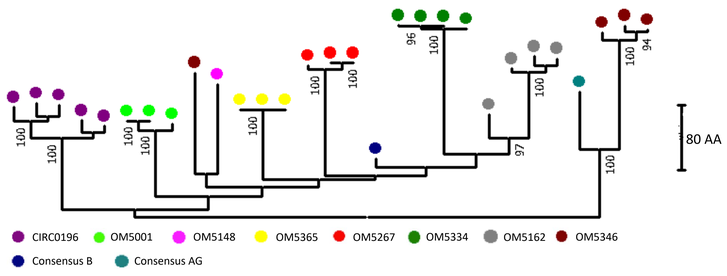


Figure 1: Maximum likelihood tree of gapstripped amino acid consensuses from each outgrowth virus. SGS was performed on 3-5 outgrowth virus per individual, with >3 sequences per QVOA well used to determine a consensus sequence. Wells containing multiple viruses (defined by the presence of >4 AA differences within sequences from the same well or the presence of fixed mutations) were excluded from the tree. Participant OM5346 was co-infected with subtypes B and AG viruses.

Figure 2: Participants with greater reservoir diversity display greater bNAb resistance

A

Participant	Average pairwise distance	Geomean IC50 for bNAbs
OM5346	0.192	4.40
OM5334	0.003	4.22
OM5365	0.000	1.42
OM5267	0.004	3.90
OM5001	0.016	1.42
OM5162	0.083	13.78
CIRC0196	0.066	13.95

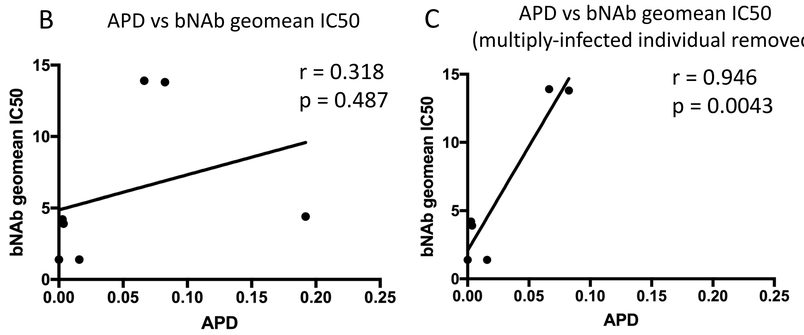


Figure 2: Average pairwise distance (APD) was calculated as a measure of reservoir diversity for each participant in the study using DIVEIN[4]. Each virus was tested for neutralization sensitivity against a panel of 10 HIV-1 bNAbs: CD4bs targeting antibodies VRC01, VRC07-523, 3BNC117, and N6, V1V2 targeting 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. Pearson correlations were performed to determine the relationship between APD and bNAb sensitivity. Interestingly, while these factors are not correlated in our data set, removing participant OM5346 (who is multiply infected with subtypes B and AG, greatly increasing APD) yielded a strong correlation (p = 0.0043). APD could not be calculated for participant OM5148 due to QVOA wells containing multiple viruses as determined by SGA.

Figure 3: Participants weakly neutralize a global panel of HIV-1 viruses

	Subtype B		Subtype C			Subtype A		Subtype BC		Subtype AE		Subtype G	
Ab Sample	TRO11	X2278	25710	CEO217	CE1176	398F1	246F3	BJOX2000	CH119	CNE8	CNE55	X1632	Geometric Mean of Neutralized Virus
Tier	2	2	1B or 2	2	2	2	2	2	2	2 or 3	2	2	
OM5346	23.0	45.6	>50	14.8	38.8	15.0	>50	43.7	>50	>50	>50	18.4	25.6
OM5148	8.11	>50	>50	49.7	38.7	15.3	49.5	>50	>50	>50	16.6	11.5	21.7
OM5334	42.1	40.7	>50	47.4	>50	18.4	49.7	>50	>50	>50	>50	>50	37.5
OM5365	10.2	46.9	>50	12.0	>50	15.5	>50	>50	>50	48.8	21.0	46.1	23.7
OM5267	35.9	44.5	45.3	46.6	19.5	15.3	45.1	>50	>50	>50	22.5	15.6	29.3
OM5001	>50	10.5	14.8	46.9	36.8	17.1	10.8	>50	>50	46.3	45.7	33.3	24.8
OM5162	23.5	9.65	>50	46.7	19.1	17.7	36.9	20.5	>50	45.5	35.8	17.6	25.0
CIRC0196	21.7	8.78	>50	>50	30.9	8.33	10.8	18.9	>50	18.8	18.1	32.7	16.6

IC50 (µg/ml): >50 | 10-50 | 1-10 | 0.1-1 | <0.1

Figure 3: IC_{50}s are calculated for each participant's Ig sample against each of the 12 global panel viruses[4]. IC_{50}s exceeding 50µg/mL are considered non-neutralizing. Percent of viruses neutralized is calculated for each sample, and geometric mean IC_{50} is calculated only from neutralized viruses.

Figure 4: All participants exhibit IgG titers against gp41 and gp120

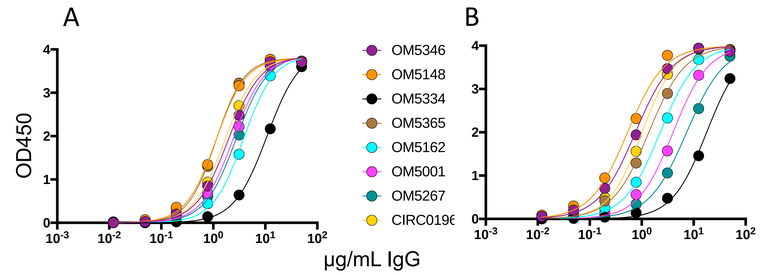


Figure 4: Participant plasma was tested for binding against 2µg/mL subtype B gp41 (A) and gp120 (B). Experimental controls consisted of mAbs 10E8 (A) and VRC01 (B), with anti-flavivirus mAb 4G2 serving as a negative control in both experiments (not shown).

Figure 5: Participants exhibit variation in autologous neutralization

Ab Sample	Reservoir virus #1	Reservoir virus #2	Reservoir virus #3	Reservoir virus #4	Reservoir virus #5
OM5346	25.4				
OM5148	26.4	29.6	16.4	10.3	13.0
OM5334	13.4	15.3	23.6	32.6	>50
OM5365	>50	>50	>50		
OM5162	39.1	>50	>50		
OM5001	28.5	>50	>50	>50	>50
OM5267	40.9	>50	>50	>50	
CIRC0196	>50	>50	>50	>50	>50

Figure 5: IC50s are calculated for each plasma sample against outgrowth virus derived from the autologous reservoir. Between 1 and 5 viruses for each individual were tested. Two individuals were able to neutralize all autologous viruses tested, four were able to neutralize a portion, and two were able to neutralize none. Interestingly, no participants exhibited potent neutralization of any autologous reservoir viruses. IC50s >50 are considered non-neutralizing, and blank spaces in the table denote where individuals had fewer than 5 viruses available for neutralization assays.

Conclusions

- SGS revealed differences in reservoir diversity between participants, with some individuals harboring unique viruses while others harbor a few clonal strains.
- Reservoir diversity was correlated with bNAb resistance when accounting for multiply-infected individuals.
- All participants exhibited robust binding titers to HIV Env protein and neutralization titers against heterologous virus.
- Isolated Ig from participants neutralized on average 8/12 global panel viruses, but neutralization was not potent.
- Participant Ig did not potently neutralize autologous virus, suggesting autologous antibodies are ineffective at neutralizing the inducible reservoir.

References and Acknowledgements

[1] Brumme *et al., AIDS,* 2019. [2]Moir and Fauci, *Immunol Rev.*, 2017. [3]Ren *et al., Journal of Virology*, 2018. [4]deCamp *et al., Journal of Virology, 2014.* [4]Deng *et al., Biotechniques*, 2010.

We would like to thank and acknowledge the BELIEVE collaboratory and the DC CFAR for funding this project. Andrew Wilson is a Ph.D candidate in the Microbiology and Immunology program of the Institute for Biomedical Sciences at the George Washington University. This work is from a dissertation to be presented to the above program in partial fulfillment of the requirements for the Ph.D degree.