HIV-1 Envelopes from Persistent Viremia on ART Show Reduced Antibody Sensitivity

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Introduction

Despite ART adherence, non-suppressible viremia (HIV RNA >20 copies/ml) persists in some donors, arising from large, infected cell clones. The mechanisms by which these clones escape immune responses is not defined, but envelope (Env) resistance to antibodies (Abs) could contribute. Therefore, we assessed the Ab neutralization sensitivity of HIV-1 Envs from 5 individuals on ART with non-suppressed viremia despite therapeutic drug levels and no evidence of drug resistance to the current ART regimen.

Methods

- Single genome sequencing (SGS) used to amplify and sequence full-length HIV-1 Env from plasma of 5 donors with non-suppressible viremia (HIV RNA >20c/mL)
- HIV-1 Env amplicons were ligated into mammalian expression vector (3.1D/V5-His-TOPO) and expressed as pseudoviruses (PSV) from HEK293T cells
- PSVs used in a luciferase-based assay against a panel of 16 monoclonal antibodies (mAbs) to measure neutralization sensitivity
- mAbs included: VRC01, 3BNC117, b12, LSEVh-ls (CD4 Binding Site or CD4bs), PG9, PG16, PGT145 (V1V2 Apex), 2G12 (Glycan), PGT-121, 10-1074, PGT-128 (V3-Glycan), PGT-151 (gp120-gp41 interface), 10e8, 4e10, and 2F5 (membrane-proximal external region of gp41) (MPER), and N6/PGDM1400x10E8 (tri-specific antibody directed to the CD4bs, V1V2 apex, and MPER binding sites

Results

- 8 unique HIV-1 Envs from 5 donors (R-09, C-03, C-02, T-13, F-07) tested (**Figure 1**)
- In general, the HIV-1 Envs tested were more resistant to Ab neutralization than a tier 1 (6535) or a tier 2 (TRO11) subtype B PSV (**Table 1**).
- HIV-1 Envs were more resistant to CD4bs, Gp41, and Apex mAbs
- Donor R-09 PSVs showed resistance to Apex mAbs and VRC01 (**Figure 2**)
- PSV R-09_C2 was resistant to the Tri-Specific mAb (N6/PGDM1400x10E8)
- Generally, HIV-1 Envs more sensitive to V3-glycan mAbs than other mAbs
- gp41 mAb 10e8 potently neutralized all the PSVs (**Table 1, Figure 2)**
- Additional potential N-linked glycosylation sites (PGNS) and changes in V5 reduce sensitivity to VRC01 (**Figure 3**)

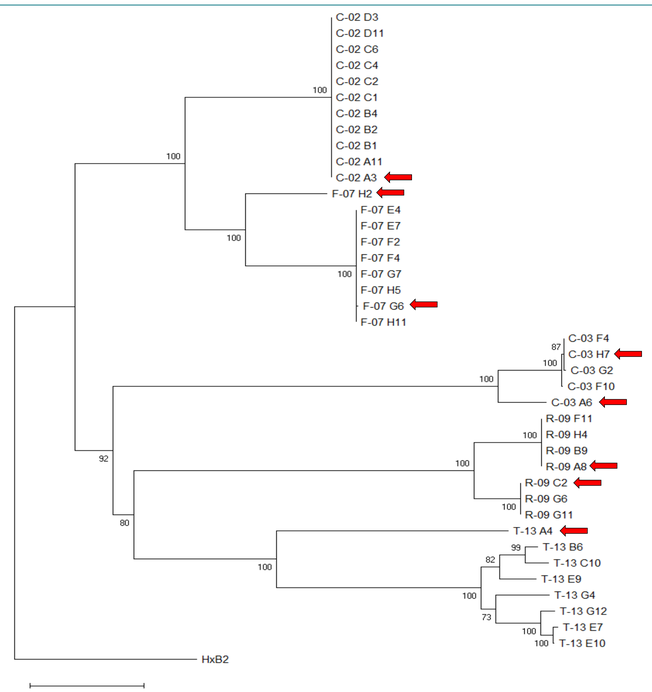


Figure 1: Phylogenetics of HIV-1 Env sequences. Neighbor-Joining method was used to infer evolutionary relationships among 40 Env sequences from plasma in donors C-02, C-03, F-07, R-09, and T-13 (rooted to the HxB2 HIV-1 Env). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths (sum of branch length = 0.45828810) in the same units as those of the evolutionary distances. Evolutionary distances were computed using the p-distance method. There were a total of 2714 positions in the final dataset. Red arrows (⟸) show unique HIV-1 Envs. Evolutionary analyses were conducted in MEGA X.

Results Continued

Table 1. Neutralization sensitivity of plasma-derived HIV-1 envelopes from donors with residual viremia on ART

	Tri-Specific	CD4bs				Apex (V1/V2)			V3-Glycan			gp41			gp120-gp41
	N6/PGDM 1400x10E8	LSEVh-LS	3BNC117	VRC01	b12	PG9	PG16	PGT-145	PGT-128	PGT-121	10-1074	4E10	10e8	2F5	PGT-151
R-09_A8*	0.61	0.70	1.58	>2.5	>2.5	>2.5	>2.5	>2.5	0.85	0.28	1.02	0.85	0.05	>2.5	>2.5
R-09_C2	>2.5	>2.5	2.50	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	0.30	2.00	>2.5	0.40	>2.5	>2.5
C-02_A3*	0.45	>2.5	0.16	1.06	>2.5	>2.5	>2.5	0.36	0.11	0.23	0.40	1.27	1.27	>2.5	0.04
F-07_G6*	0.30	0.60	0.19	1.43	>2.5	>2.5	0.24	0.17	0.08	0.05	0.05	>2.5	0.10	>2.5	0.02
F-07_H2	0.06	0.31	0.04	0.22	>2.5	0.04	0.04	0.01	0.05	0.02	0.05	>2.5	0.28	1.38	0.01
C-03_A6	0.23	2.5	0.22	1.44	0.77	0.78	>2.5	0.11	>2.5	>2.5	>2.5	2.5	0.21	2.23	>2.5
C-03_H7*	0.21	0.31	0.12	1.1	>2.5	0.37	2.08	0.08	>2.5	>2.5	0.34	0.70	0.11	0.85	>2.5
T-13_A4	0.09	0.41	0.34	1.40	>2.5	2.5	2.5	>2.5	2.5	0.89	1.00	>2.5	0.76	>2.5	0.02
6535	0.09	0.11	0.46	1.11	>2.5	0.31	>2.5	>2.5	0.02	0.005	0.02	1.06	0.08	>2.5	0.01
TRO11	0.20	>2.5	0.06	0.56	>2.5	>2.5	>2.5	0.09	0.05	0.03	0.05	1.60	0.1	>2.5	>2.5
PVO	0.70	1.34	0.14	0.73	>2.5	>2.5	-	0.58	0.04	>2.5	0.19	>2.5	1.13	>2.5	0.06

IC_{50} (μg/ml) 0.01 — 2.5

*** HIV-1 Env sequences known to match proviral clonal sequence**

- PSVs incubated for 1h with mABs
- PSV/Ab mixtures added to target cells
- Incubated for 3 days
- Cells lysed and assayed for luciferase activity
- Percentage neutralization was calculated relative to cell-only and virus-only controls
- PSVs 6535, TR011, and PVO used as controls
- IC_{50} values of >2.5μg/ml are considered resistant

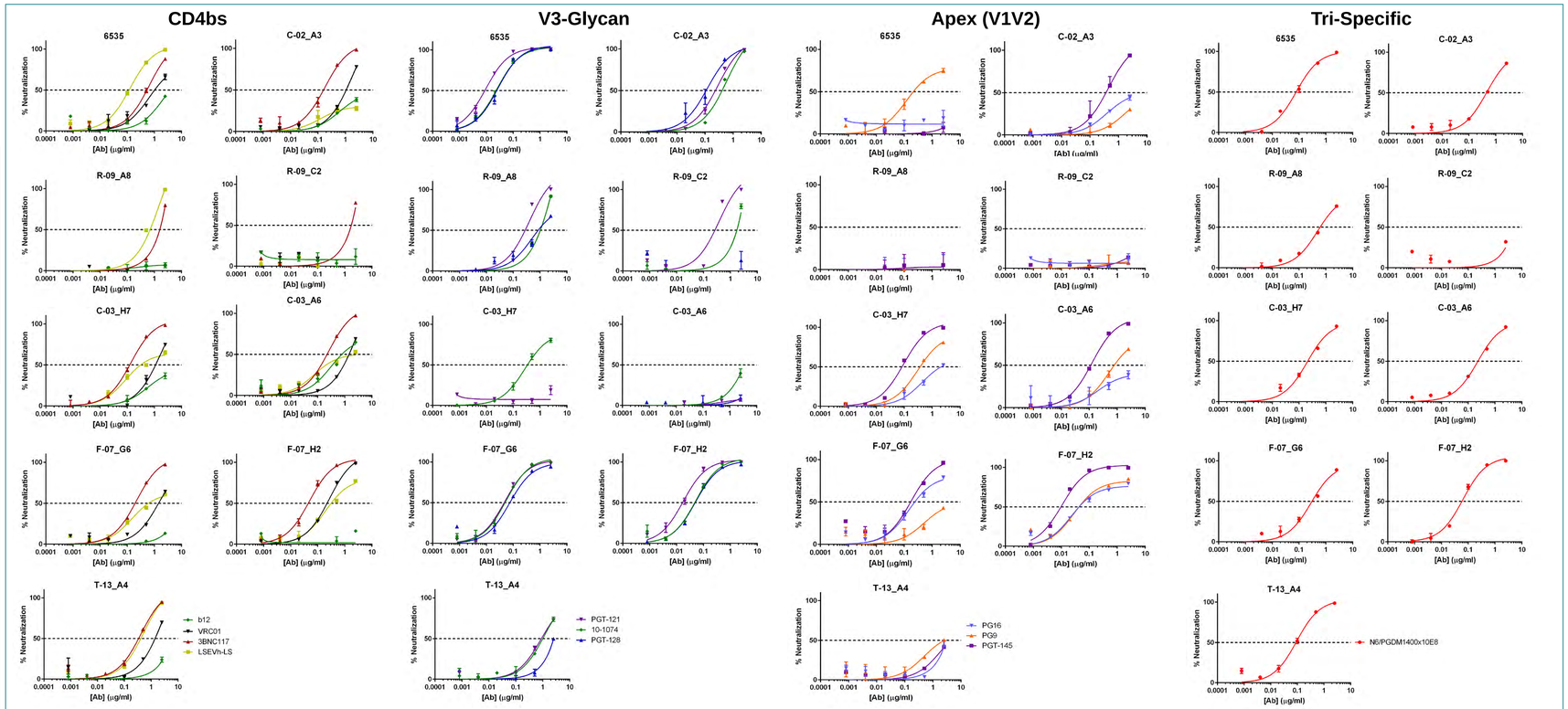
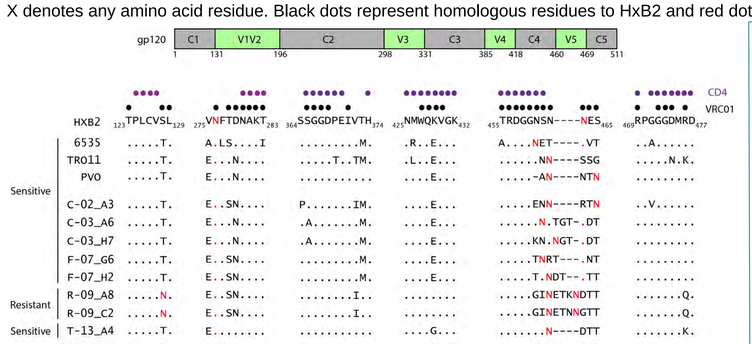


Figure 2: Donors with residual viremia on ART carry neutralization resistant Env sequences. Donors C-02 (C-02_A3), C-03 (C-03_H7 and C-03_A6), F-07 (F-07_G6, F-07_H2), R-09 (R-09_A8 and R-09_C2), and T-13 (T-13_A4) PSVs were tested against mAbs targeting CD4bs (b12, VRC01, 3BNC117, LSEVh-LS), V3-Glycan (PGT-121, 10-1074, PGT-128), V1V2 Apex (PG16, PG9, PGT-145), Gp41 membrane-proximal external region (MPER) (4E10, 10e8, 2F5), the Gp120-Gp41 interface (PGT-151) or the tri-specific Ab (N6/PGDM1400x10e8) directed to the CD4bs, V1V2 apex, and MPER. Percentage neutralization was calculated relative to cell-only and virus-only controls. The tier 1b subtype B PSV 6535 is used as a neutralization control. Dashed line corresponds to 50% neutralization.

Figure 3: An additional potential N-linked gylcosyltion site (PNGS) and changes to V5 modulate VRC01 neutralization. A schematic of gp120 at the top with variable regions V1 to V5 in green and the conserved regions C1 to C5 in grey; regions are numbered according to HxB2. The protein sequence of the CD4bs and VRC01 epitope was analyzed for donor HIV-1 Env sequences (C-02, R-09, C-03, F-07, T-13) along with control HIV-1 Envs: 6535 (tier 1b), TRO11 (tier 2) and PVO (tier 3). VRC01 and CD4 contact residues are denoted with black and purple circles respectively. Asn residues highlighted in red are PNGS with the motif N-X-T/S where X denotes any amino acid residue. Black dots represent homologous residues to HxB2 and red dots represent Asn PNGS residues conserved with HxB2. Dashes denote regions that tolerate amino acid insertions.



Conclusions

- HIV-1 envelopes derived from residual viremia on ART are generally more resistant to Ab neutralization compared to tier 1 (6535), tier 2 (TRO11) or tier 3 (PVO) subtype B Env
- Donor R-09 HIV-1 envelops (A8 and C2) are resistant to most mAbs tested
- Donor R-09 HIV-1 envelops (A8 and C2) are resistant to VRC01, likely due to an additional N-glycan site at a VRC01 contact site and extended V5 regions
- Most of the donor HIV-1 envelops were sensitive to at least two out of three V3-glycan Abs, except for C-03_A6 which was resistant to all three
- mAbs 3BNC117 and 10e8 neutralized all donor viruses, indicating therapeutic potential for clearing persistent viremia in the individuals studied