DISTINCT HIV-1 RESISTANCE PROFILES AGAINST BNAB IN INTACT VS DEFECTIVE VIRAL GENOMES

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Abstract 00180

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Background

Broadly neutralizing antibodies (bNAbs) targeting HIV-1 Env are under study as a strategy in cure research. Viral *env* is genetically diverse, with distinct bNAb sensitivity profiles across subtypes and across hosts. We analyzed and identified bNAb-associated resistance genotypes in intact and defective HIV-1 DNA genomes and **hypothesize that intact genomes will have distinct bNAb resistance profiles relative to defectives.**

Methods

HIV-1 DNA genomes were sequenced from 1 viremic and 14 virologically suppressed individuals on long-term ART via single genome amplification (HXB2 638-9632). Genomes were classified as intact or defective using HIVSeqinR software. Resistance residues against CD4 binding site-, V2-, and V3-targeting bNAbs, and hypervariable loop characteristics such as total length, number of N-linked glycosylation sites (PNGs), and electric charge were assessed.



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Results

Figure 1. All HIV-1 *env* associated with genome-intact proviruses were genetically unique compared to *env* associated with defective genomes.

The maximum likelihood phylogenetic tree contains 137 *env* sequences from 15 donors. Leaves are color-coded by donor and branches are color coded by subtype (dark blue for clade B, dark green for clade CRF01-AE, and purple for the two donors infected by a recombinant virus). One donor (D5) had both B clade and recombinant genomes. Different tip symbols identify *env* from either intact genomes (filled circles), defective genomes (filled triangles), premature stop genomes (open triangle), or inferred intact (filled square). Color-coded bars at the tips indicate different sampling time points: dark gray for the first time point and lighter gray for the second time point sampled from the same donor.

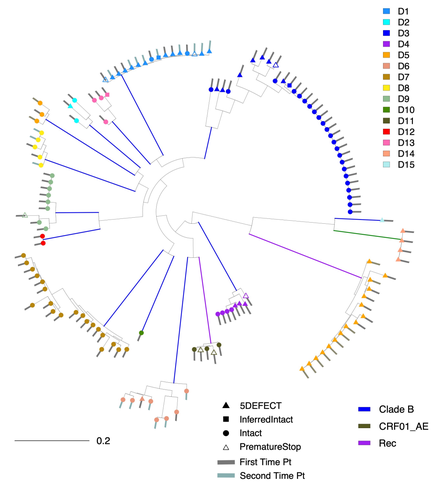


Figure 2. Each of the 15 study donors harbored distinct viral Env with unique bNAb-resistance profiles.

Logo plots of 54 V3-bNAb neutralization sensitivity signature sites for 137 Env from 15 donors revealed each donor have different bNAb-resistance profiles. Env from intact genomes are shown on the left and those from 5′ psi defective genomes on the right as representative examples. Residues font sizes are proportional to the frequencies found in each sequence set. Red font indicates predicted association with resistance, blue with sensitivity, or black if indetermined. Top row shows HXB2 residues at those sites. Blanks indicate positions where Env carries the same residue as HXB2. Donor names (D1-D15) are color-coded according to subtype. Five donors (D7-D13) had Env from intact genomes only, while two donors (D14 and D15) had Env from defective genomes only. N/D stands for not detected.

Figure 3. In Donor 7, we detected two distinct bNAb-resistance profiles among genome-intact proviruses.

(A) Env positions where Env from viremic donor D7 differ. The resistant genotype carries glycosylation sites at HXB2 positions 130 and 334, associated with V2- and V3-bNAb resistance respectively. A subset of Env from the resistant genotype lineage carries residue H at position 330, which is associated with V3 bNAb sensitivity, and are compensate with the loss of glycan at position 295, where the glycan is associated with V3 bNAb sensitivity. Additionally, in the V1V2 region combined, the resistant genotype has statistically significantly longer hypervariable loops, lower V1V2 charge, and higher number of glycosylation sites (p=0.004, 0.002 and 0.002 respectively by permutation test). Longer V1V2 loops and higher number of PNGs in V1V2 are both characteristics associated with V3-bNAb resistance. Three Env in the sensitive group and one in the resistant group that show mixed profiles were confirmed to be statistically significant recombinants via RAPR and are highlighted in bold ($p=2.4\times10^{-7}$, 9.7×10^{-6}, 4.8×10^{-10}, and 7×10^{-3} respectively). (B) Recombination events between the sensitive and the resistance haplotypes detected by RAPR.

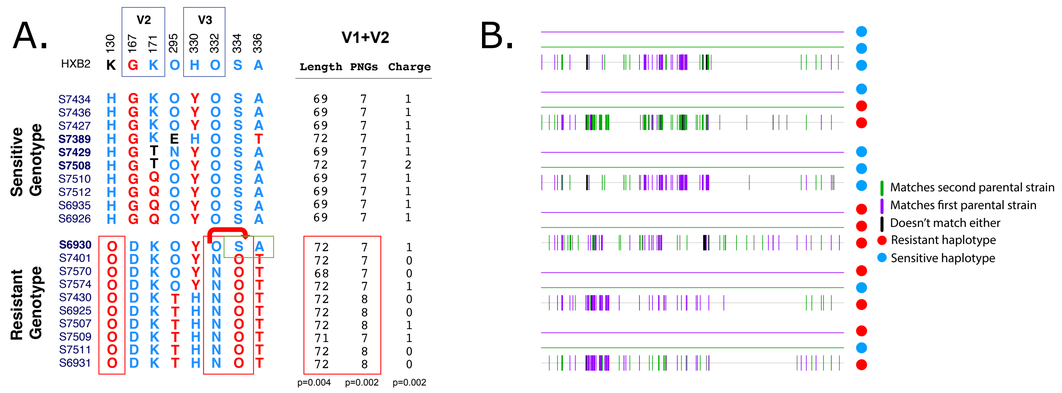
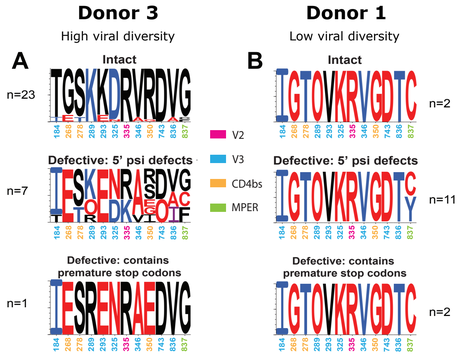


Figure 4. Distinct resistance profiles against bNAbs in intact vs defective proviral genomes were detected in donor 3, but not in donor 1.

Logo plots from two donors at the high and low end of the diversity spectrum are shown here. On the left (panel A), defective genome Env (bottom two panels) from donor D3 had significantly more amino acid signatures associated with bNAb resistance compared to intact genome Env (top panel; $p<0.0006$). Logo plots of these sites (color coded accordingly to the bNAb class they were associated with) show that only 5 out of 23 (22%) intact genome Env carried residues associated with bNAb neutralization resistance, compared to all of the defective genome Env. By contrast, donor D1 (panel B) showed overall very low diversity in all Env, from both intact and defective genomes (shown here logo plots of the same set of sites as donor D3 for comparison).



Conclusions

Our results reveal that resistance profiles across all bNAb classes in *env* region are diverse across hosts and can be distinct between intact and defective genomes within host. Our results highlight the importance of understanding genome-intact provirus resistance profiles when screening individuals for enrollment in bNAbs clinical trials.