Enhanced antibody recognition against HIV resistant strains by directed evolution

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Background

Human Immunodeficiency Virus (HIV) is responsible for 1.5 million new infections and 650,000 related deaths worldwide annually. There is an urgent need to develop efficacious prevention and treatment options against HIV. Passive immunization with broadly neutralizing antibodies (bNAbS) is one promising approach to prevent infection, but enhanced potency and recognition of broad HIV-1 variants continues to be a challenge for the field. VRC07-523LS FR3-03 is an anti-HIV-1 bNAb that targets the CD4-binding site on the HIV-1 envelope glycoprotein. This antibody exhibited high potency (median IC50 = 0.065 µg/mL) and breath (neutralized 97% with IC50 < 50 µg/mL) against a panel of 208 HIV strains.

Figure 1: Number of deaths from HIV/AIDS per 100,000 people worldwide in 2019. (Institute for Health Metrics and Evaluation, 2021 - https://ourworldindata.org/hiv-aids.)

Goal

We applied in vitro antibody engineering techniques to enhance affinity of VRC07-523LS FR3-03 against resistant strains, and consequently improve the neutralization potency and breadth of this antibody.

Methods

Using site-saturation mutagenesis (SSM), DNA libraries were generated encoding all possible amino acid substitutions or deletions for each residue in the VRC07-523LS FR3-03 heavy and light variable-region template genes. The resulting antibody libraries were cloned into a yeast surface display. The Fab libraries were then expressed on the surface of yeast for functional evaluation through fluorescence-activated cell sorting (FACS) against BG505 and the trimers of six HIV-1 strains to which the template antibody is resistant – AC.6540, AC.6545, AE.620345, AG.T278-50, B.BL01, and C.DU172.17, followed by in silico antibody functional interpretation of the Next Generation Sequencing (NGS).

Figure 2: Directed evolution was used to optimize the mAb VRC07-523LS FR3-03 for enhanced binding to HIV antigens: (A) Substitution and deletion SSM libraries were designed for VH and VL regions of the anti-HIV bNAb VRC07-523LS FR3-03. (B) SSM libraries were cloned into yeast display and screened by FACS to determine the impact of each possible single mutation. (C) Sorted sequences are sequenced by NGS, and bioinformatic analyses are used to interpret the functional impact of each mutation. (D) Selected variants are characterized for neutralization activity, binding affinity, and structural mechanisms (Adapted from Madan, PNAS, 2021).

Figure 3. Representative FACS gating strategy for functional screening. Yeast cells were stained with anti-FLAG-APC and either A.BG05-DS-SOSIP (6 nM) AC.6540 (6 nM), AC.6545 (50nM), AE.620345 (50 nM), AG.T278-50 (50 nM), B.BL01 (50nM), or C.DU172.17 (50 nM) conjugated to Streptavidin-Phycocerythrin (SA-PE).

Figure 4: Single mutations enable improvements in recognition against A.BG0505 . VRC07-523LS FR3-03 SSM libraries were screened for two rounds against BG505-DS-SOSIP showing enhanced affinity in Round 2, when compared to the wild-type template.

Figure 5: Single mutations in variable region genes enhanced recognition to resistant strains. After two rounds of enrichment with BG505-DS-SOSIP, the VRC07-523LS FR3-03 SSM mutated libraries were screened against six resistant probes. Enhanced binding performance in the sorted libraries was observed against all resistant probes, except for B.BL01, revealing clear phenotypic differences from the template antibody.

Conclusion

These data demonstrate that SSM combined with yeast display and next-generation sequencing is a powerful approach to improve the recognition of anti-HIV monoclonal antibodies against resistant strains, including potent antibodies that target the vulnerable CD4 binding site epitope.

Next steps

Precision antibody selection with NGS and bioinformatics, expression, and biochemical and functional characterization of enhanced mutants.

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