Resistance of inducible, infectious HIV-1 to autologous neutralizing IgG

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BACKGROUND

- The major barrier to HIV-1 cure is the latent reservoir in resting CD4+ T cells.
- Rapid, exponential viral rebound occurs when antiretroviral therapy (ART) is stopped. Cure strategies aim to prevent rebound.
- We showed autologous neutralizing antibodies (aNAbs) can prevent a substantial but variable fraction of outgrowth of some reservoir viruses (Bertagnolli et al., PNAS 2020).

Goal

We compared aNAb activity of reservoir viruses in 28 people with HIV (PWH) on ART from 4-26 years. We analyzed factors that may be responsible for the variable resistance to aNAb neutralization, investigated if aNAbs persist over time on ART, and explored whether there is antibody-based immune selection against reservoir cells carrying viruses recognized by aNAbs.

METHODS

We performed **quantitative viral outgrowth assays** using resting CD4+ T cells of PWH on ART. Next, **single genome sequencing** of *env* was done on outgrowth positive supernatants (Fig. 1A) to **generate pseudoviruses** with the *env* sequences of inducible, replication-competent proviruses. Pseudoviruses were tested in TZM.bl-based **neutralization assays** incorporating purified autologous IgG (Fig. 1B). To evaluate immune selection, **ADCC assays** were performed with target cells infected with participant-derived pseudoviruses, NK cells, and autologous IgG (Fig. 1 C).

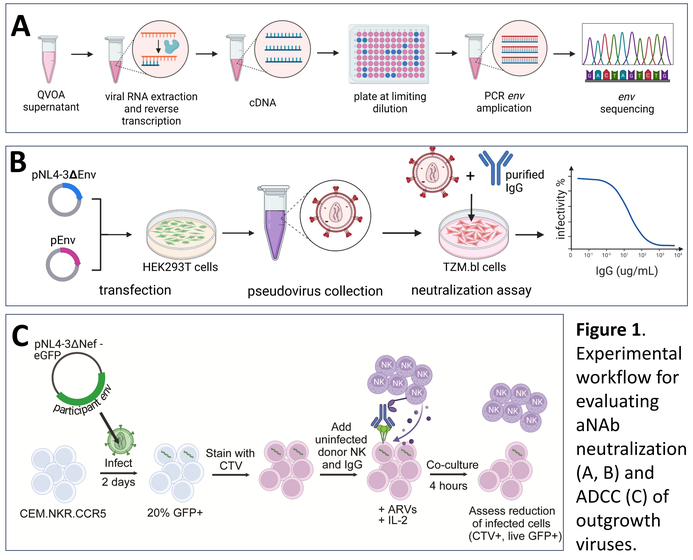


Figure 1. Experimental workflow for evaluating aNAb neutralization (A, B) and ADCC (C) of outgrowth viruses.

Majority of outgrowth viruses are resistant to autologous neutralizing antibodies

RESULTS

Most outgrowth isolates from PWH on ART were resistant to neutralization by aNAbs with IC50 values >100 µg/ml.

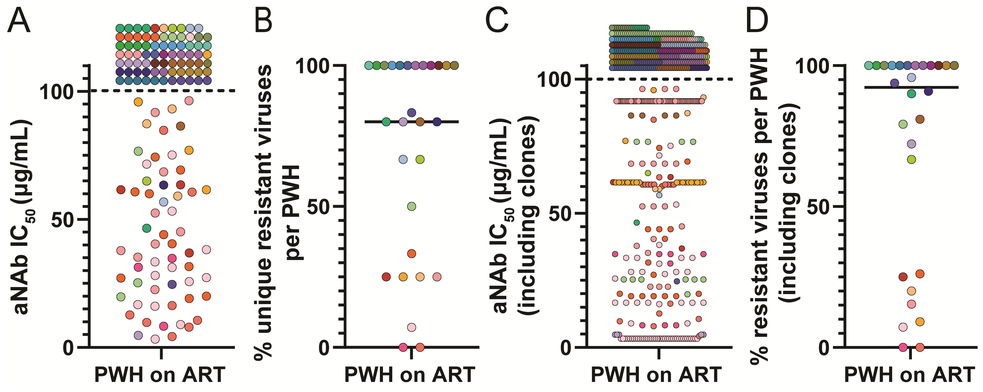
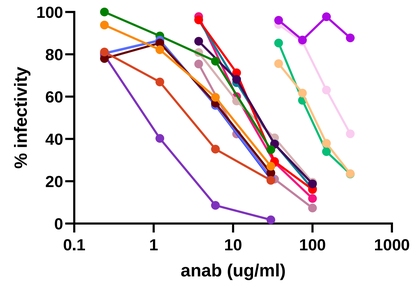


Figure 2. (A) aNAb IC50 values were determined in neutralization assays for unique pseudoviruses generated from outgrowth viruses from PWH on ART. Autologous IgG concentrations up to 100 µg/ml were used. Each circle represents one unique viral isolate, and each color represents one participant. (B) Fraction of resistant viruses among the total number of viruses tested in each PWH expressed as a %. Bar represents median. (C) Data from unique isolates shown in (A) with additional data points representing independent isolates from the same PWH with *env* sequences identical to those shown in (A). (D) Percentage of outgrowth viruses resistant to neutralization (IC50 >100 µg/ml) by aNAb per PWH from (C). Bar represents median.

To determine whether the low fraction of reservoir viruses neutralized by autologous IgG in PWH on ART was due to a waning of the neutralizing antibody response, we examined neutralization of SF162, a highly neutralization-sensitive tier 1 virus.



SF162 was neutralized by aNAbs isolated from 16 of 17 long-term ART participants.

Figure 5. Neutralization assays were conducted with SF162 virus in the presence of IgG isolated from long-term ART participants at concentrations up to 300 µg/ml. Each colored line represents data from one participant.

Longitudinal analysis showed stability and waning of antibody titers over time.

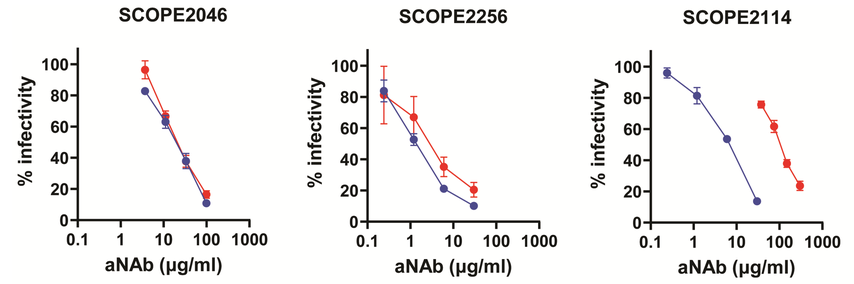


Figure 6. Neutralization assays against autologous outgrowth viruses were conducted in the presence of aNAbs isolated at different timepoints from PWH with largely resistant reservoirs.

Cells infected with outgrowth viruses were susceptible to ADCC with autologous IgG after long-term ART.

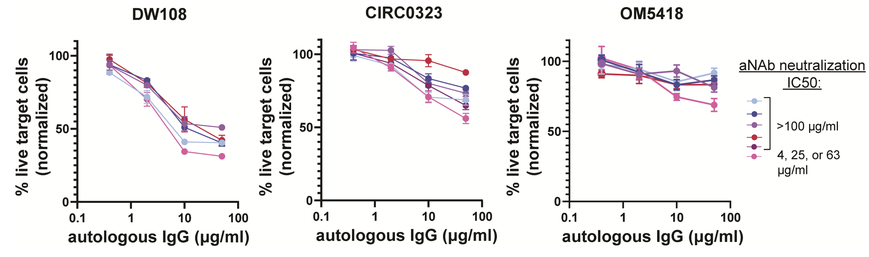


Figure 7. ADCC assays for PWH with largely aNAb-resistant reservoirs after long-term ART. Each color represents one outgrowth virus. Data represented as % live CTV+ GFP+ target cells.

Many outgrowth viruses from PWH with aNAb-resistant reservoirs remained sensitive to bNAbs.

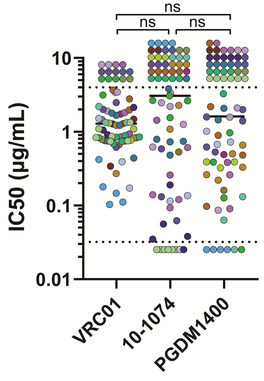
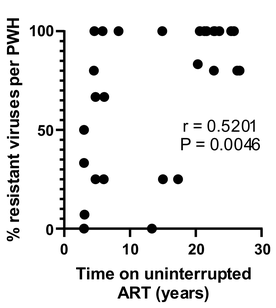


Figure 3. (A) bNAb IC50 values were determined in neutralization assays for unique pseudoviruses generated from outgrowth viruses from aNAb-resistant group of PWH on ART. Concentrations between 0.032-4 µg/ml were used. Each circle represents one unique viral isolate, and each color represents one participant. Bar represents median.

aNAb-resistance positively correlated with time on uninterrupted ART.

Figure 4. Data from Fig. 1B replotted against time on uninterrupted ART.



CONCLUSIONS

- Many outgrowth viruses that persist are resistant to neutralization by aNAbs. This may reflect gradual Ab-mediated immune selection and/or waning of Ab titers.
- Many aNAb-resistant outgrowth viruses were sensitive to bNAbs.
- aNAbs persist after >20 years and remain functional.

ACKNOWLEDGEMENTS

Thank you to all our study participants and funding sources.

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