The fraction of HIV reservoir variants neutralized by autologous IgG correlates with time to rebound

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482

Abstract

Introduction

- Latently-infected resting memory CD4+ T cells, known as the latent reservoir, represent the major barrier to cure.
- During untreated infection, HIV-1 continuously evolves to evade autologous neutralizing antibody (anAb) responses, which results in little to no contemporaneous neutralization.[3-4]
- Antiretroviral therapy (ART), however, reduces viremia to undetectable levels by most clinical assays and halts viral evolution, but is not curative. If ART is stopped, rapid and exponential viral rebound occurs.
- The principal goal of HIV-1 cure research is to prevent viral rebound. However, among people who stop ART, it is difficult to predict 1) which isolates may contribute to initial viral rebound and 2) when it might occur.
- The ability to predict both features prior to ART interruption may be necessary towards efforts aiming for a functional HIV cure.

Hypothesis

We hypothesize that contemporaneous anAbs influence viral rebound dynamics. anAb-sensitive reservoir isolates detected prior to ART interruption may not rebound and the aNAb-resistant isolates may be genetically similar to virus that rebounds.

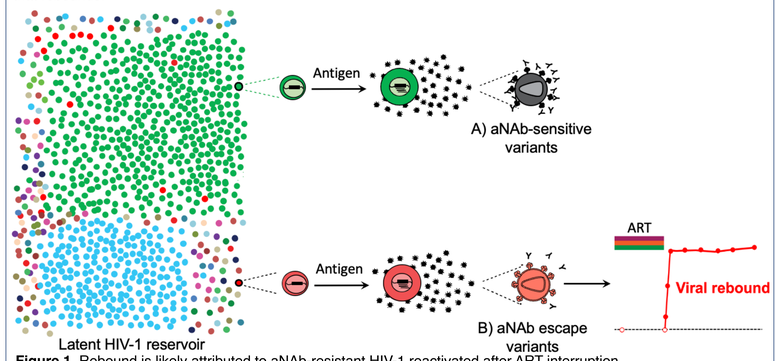


Figure 1. Rebound is likely attributed to aNAb-resistant HIV-1 reactivated after ART interruption.

Methods Modified quantitative viral outgrowth assays (QVOAs) were conducted using participant-derived resting CD4+ T cells and aNAbs collected prior to ART interruption. QVOAs were conducted in the presence of either: no IgG, HIV- donor IgG, or contemporaneous autologous IgG antibodies. See methods section for more details.

Results and Conclusions

We have identified the percentage of inducible, replication-competent reservoir isolates sensitive to neutralization by contemporaneous aNAbs prior to the ATI has a statistically significant correlation with time to viral rebound. Furthermore, phylogenetic analyses revealed that aNAb-resistant reservoir variants were genetically similar to rebound virus in multiple participants, and in Tzm-bl direct neutralization assays were highly resistant.

Methods

Study Participants

Adults with HIV-1 on suppressive ART were enrolled at University of California – San Francisco (UCSF) for the Gates-SCOPE Analytical Treatment Interruption (ATI) trial. Selection criteria included viral suppression for > 6 months and undetectable plasma HIV-1 RNA levels (< 20 copies per mL) prior to the ATI. Participants underwent leukapheresis under a protocol approved by UCSF and SCOPE Institutional Review Board (IRB). All participants provided written informed consent. Coded, deidentified leukapheresis products were shipped by same-day courier to the Johns Hopkins School of Medicine for processing and analysis under a protocol approved by a Johns Hopkins University School of Medicine IRB.

Modified Quantitative Viral Outgrowth Assay

Highly purified resting CD4+ T cells from each SCOPE ATI participant were seeded into three QVOA culture arms at 200,000 resting CD4+ T cells/well with up to 107 replicate wells per arm (typically 321 wells per study participant). Cultures were set up with either: no IgG, HIV- donor IgG (50 µg/mL), or with contemporaneous autologous IgG (50 µg/mL). Cell cultures were maintained as previously described.[5] On day 14, culture wells were assayed for p24 antigen by HIV-1 p24 ELISA (Perkin Elmer).

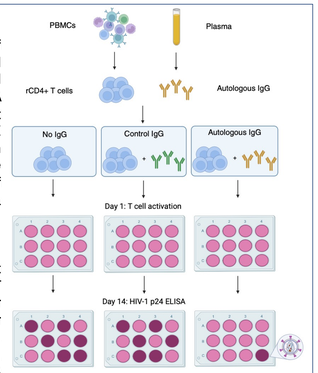


Figure 2. Modified QVOA with either no IgG, uninfected donor IgG, or contemporaneous autologous IgG.

Single Genome Env Sequencing

HIV-1 *Env* from cDNA was amplified with a two-step, nested PCR protocol. The outer PCR was completed in a volume of 10 µL containing 10x PCR buffer, 50 mM MgCl2, 10 mM PCR dNTP mix (Promega), 5 U/µL of Platinum Taq DNA Polymerase High Fidelity with a 50 µM final concentration of primers envB5out (5′TAGAGCCCTGGAAGCATCCAGGAAG 3′; nt 5853 to 5877 of the HXB2 sequence) and envB3out (5′ TTGCTACTTGTGATTGCTCCATGT 3′). PCR conditions were 94°C for 2 min (94°C for 30 s; 50°C for 30 s; 72°C for 2.5 min) for 44 rounds, and 72°C for 3 min. The nested, inner PCR was completed in a total volume of 18 µL using 2 µL of the first round PCR product diluted 1:1 with Tris: HCl, pH 8.0, and 50 µM of the primers envB5in (5′TTAGGCATCTCCTATGGCAGGAAGAAG 3′; nt 5957 to5983 of the HXB2 sequence) and envB3in (5′GTCTCGAGATACTGCTCC-CACCC 3′; nt 8882 to 8904 of the HXB2 sequence). PCR conditions were conducted at 94°C for 2 min (94°C for 30 s; 55°C for 30 s; 72°C for 2.5 min) 41 times, and finally 72°C for 3 min. PCR products were subsequently run on a 1% agarose gel for analysis. Bands at 3 kb were subjected to Sanger sequencing. Plasma viremia was sequenced by Oxford Nanopore and PacBio.

Phylogenetic Analyses

Full-length Env contigs from single genome sequencing were aligned to a consensus HXB2 sequence using the Geneious Prime Software and BioEdit. Maximum-likelihood phylogenetic trees were constructed using IQ Tree (LANL). Trees were visualized in MEGA11, and data annotations included using Adobe Illustrator.

Results

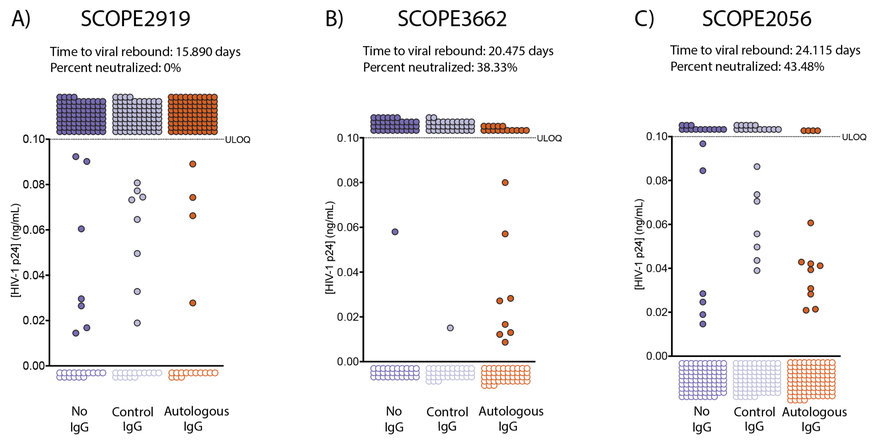


Figure 3. HIV-1 p24 ELISA results for three modified QVOA arms conducted for three representative participants: (A) SCOPE2919, (B) SCOPE3662, and (C) SCOPE2056. Neutralization by autologous IgG is reflected in the decreased p24 antigen levels in the QVOA arms with autologous IgG as compared to the two control arms with no IgG and control IgG.

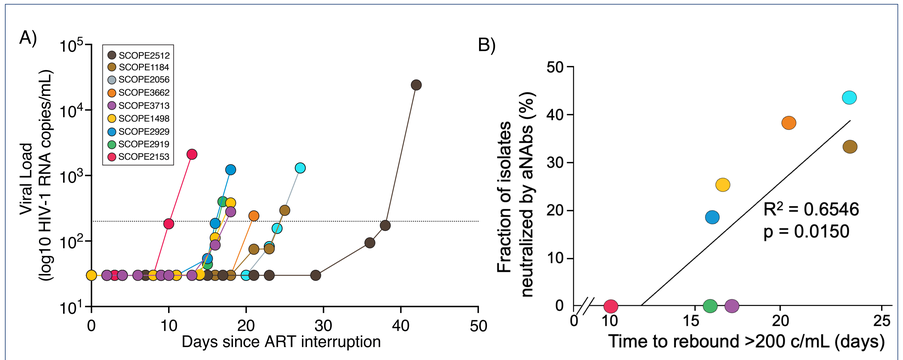


Figure 4. Rebound viremia detected in nine SCOPE ATI participants following ART interruption. (A) Variable time to >200 copies HIV-1 RNA/mL (dashed line) following ART interruption; ranges from 10-38 days. (B) The percentage of inducible, replication-competent reservoir isolates neutralized by autologous IgG in the modified QVOA has a statistically significant correlation with time to rebound.

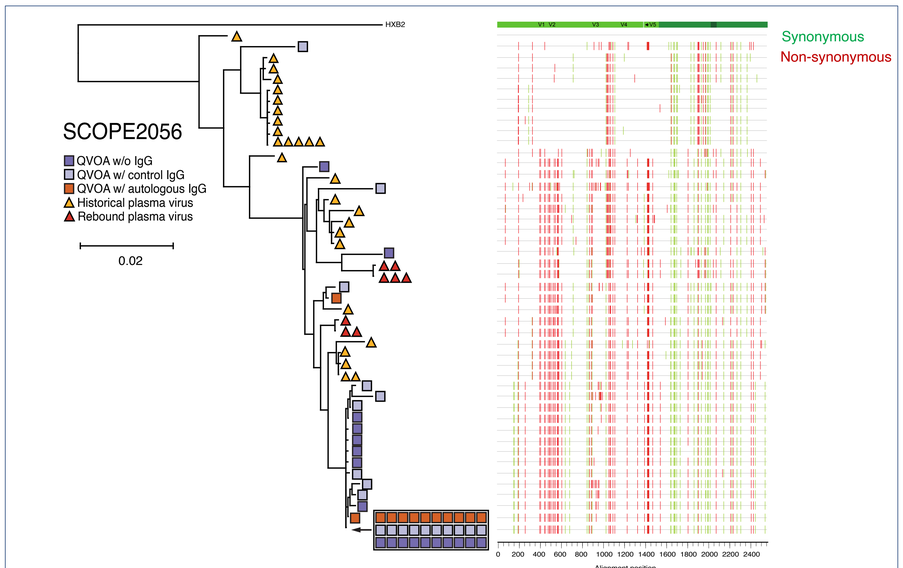


Figure 5. Phylogenetic analysis of full-length Env sequences from each experimental arm of the modified QVOA for SCOPE2056. HIV-1 outgrowth sequences indicated as dark purple, light purple and orange squares grew out in the presence of either no IgG, control IgG, or autologous IgG, respectively. There are mutations in the variable loops of Env consistent with escape from autologous neutralizing antibodies. Only isolates found in the lower part of the tree could replicate exponentially in the presence of 50 ug/mL of autologous IgG antibodies when included in the modified QVOA. These outgrowth isolates had similar escape mutations and were genetically similar to the virus that rebounds following ART interruption.

Results Cont'd

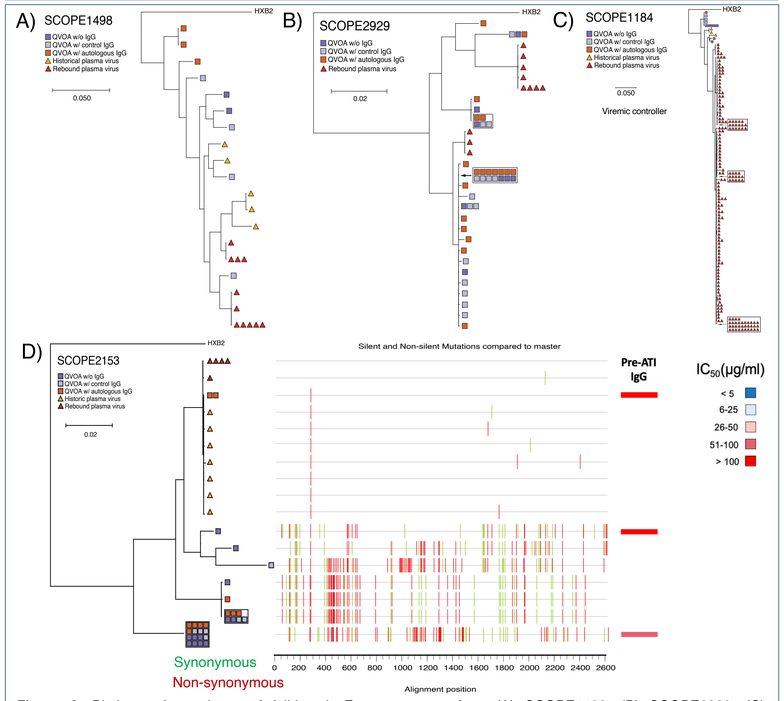


Figure 6. Phylogenetic analyses of full-length Env sequences from (A) SCOPE1498, (B) SCOPE2929, (C) SCOPE1184, and (D) SCOPE2153. Outgrowth isolates were genetically similar to rebound virus for most participants, except SCOPE1184, a known viremic controller with few outgrowth wells. In SCOPE2153, outgrowth isolates nearly identical to rebound virus were highly resistant as shown by Tzm-bl direct neutralization assays and had an IC_{50} greater than 100 ug/mL autologous IgG antibodies from the baseline ATI timepoint.

Conclusions

1. The percentage of reservoir variants neutralized by contemporaneous aNAbs at 50 ug/mL in the modified QVOA has a statistically significant correlation with time to viral rebound.
2. Autologous IgG-resistant outgrowth viruses were genetically similar to virus that rebounds following ART interruption.
3. People on ART for more than two decades had detectable viremia within 2-4 weeks following ART interruption.
4. ATI studies using time to rebound as an outcome measurement should consider the role of aNAbs.

Future Work

1. Complete modified QVOAs for additional SCOPE ATI participants and compare outgrowth with rebound sequences.
2. Complete Tzm-bl direct neutralization assays testing baseline IgG against rebound and latent variants.

References

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