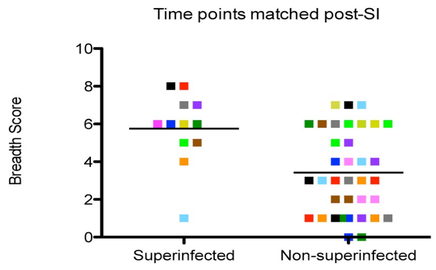
Isolation and characterization of HIV-1 superinfection neutralizing antibodies

Felicitas Ruiz[1, 2, 3], Mackenzie Shipley[1], and Julie Overbaugh[1, 4]

[1] Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA
[2] Molecular & Cellular Biology Graduate Program, University of Washington, Seattle, WA
[3]Email correspondence: fruiz@fredhutch.org
[4]Email correspondence: joverbau@fredhutch.org

Introduction

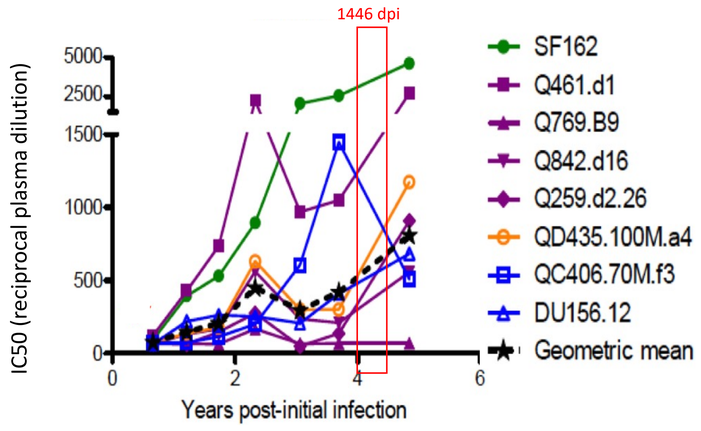
- Broadly neutralizing antibodies (bNAbs) are thought to be an important component of a protective HIV-1 vaccine as bNAbs block infection of cells by cell-free virus and prevent infection in non-human primate studies (Pegu et al., 2017, Pegu et al., 2019)
- Efforts to elicit broadly neutralizing antibody responses with vaccines have been difficult to achieve with current vaccination strategies, though these responses do develop in a subset of infected adults
- HIV superinfected (HIV-SI) individuals develop broader NAb responses compared to singly infected individuals and can develop broad NAb responses as early as ~1 year post-SI (Steward et al., 2018, Cortez et al., 2012).
- HIV-SI NAb responses appear to be polyclonal as epitope mapping studies did not detect a dominant epitope, suggesting that HIV-SI individual's may have a polyclonal response contributing to broad plasma responses
- A polyclonal response is likely more difficult for virus to escape.
- How HIV-SI develop broad NAb activity may provide insights to optimal approaches to elicit a polyclonal bNAb responses



HIV-SI: 1.68x greater breadth (p=0.001)

Adapted from Cortez et al., 2012

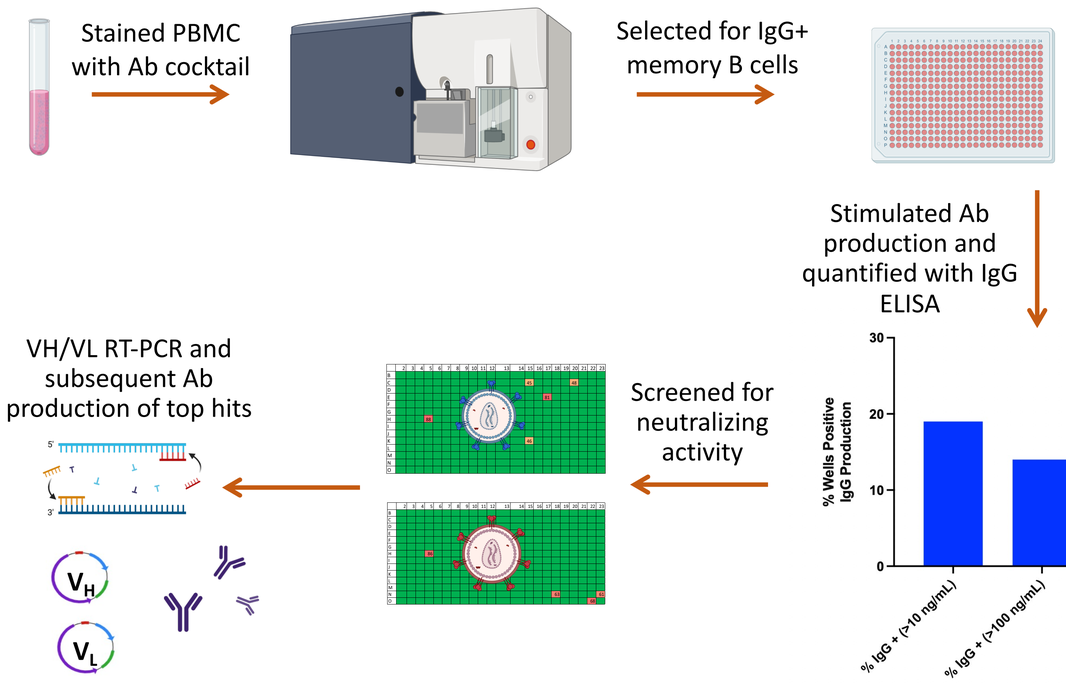
Plasma neutralizing activity of QB850



Study Goal: isolate and characterize QB850 NAbs contributing to plasma breadth

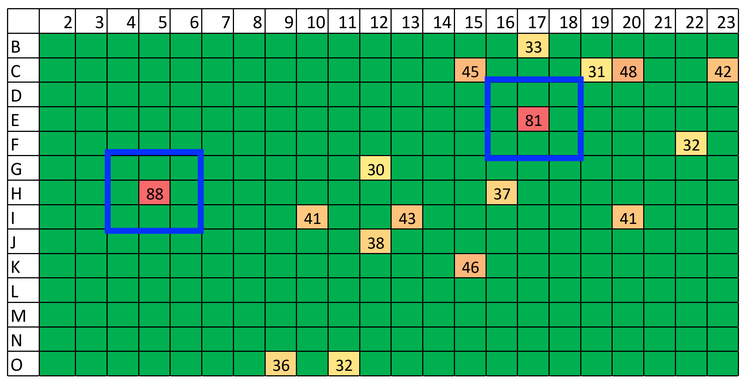
- QB850 was identified as having broad neutralizing activity
- QB850 was initially infected with a Clade A virus, and subsequently infected by a Clade D virus approximately 2 months later
- **Cross-clade and potent neutralizing activity was observed in plasma within 1 year following SI**

Approach: B cell culture and antibody cloning



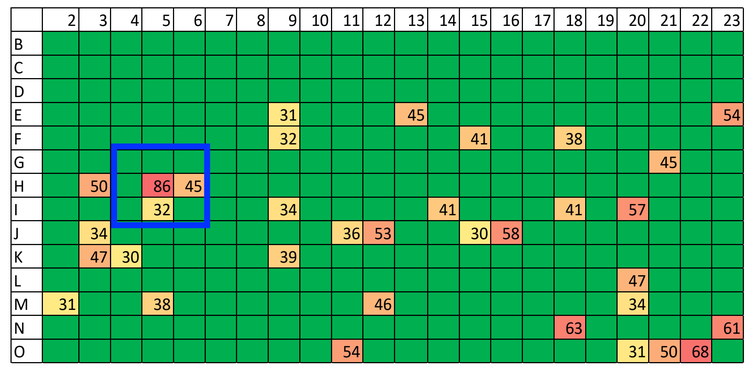
Peripheral blood mononuclear cells (PBMCs) were obtained 1446 days post HIV-infection from patient QB850. We sorted for all memory B cells ($CD19^+$, IgM^-, IgD^-) and plated at ~ 6 cells/well. IgG ELISA data was collected as an indicator of relative antibody proliferation prior to microneutralization screen. cDNA was then obtained by reverse transcription using random primers and the variable heavy and light chain genes were amplified using VH and VL-specific primer sets. Heavy and light chain genes can then be subcloned in expression vectors and co-transfected into 293F cells. The resulting antibodies can be functionally characterized by neutralization assays, ELISA binding assays, as well as for ADCC activity.

Microneutralization screen identifies potential HIV-specific neutralizing samples

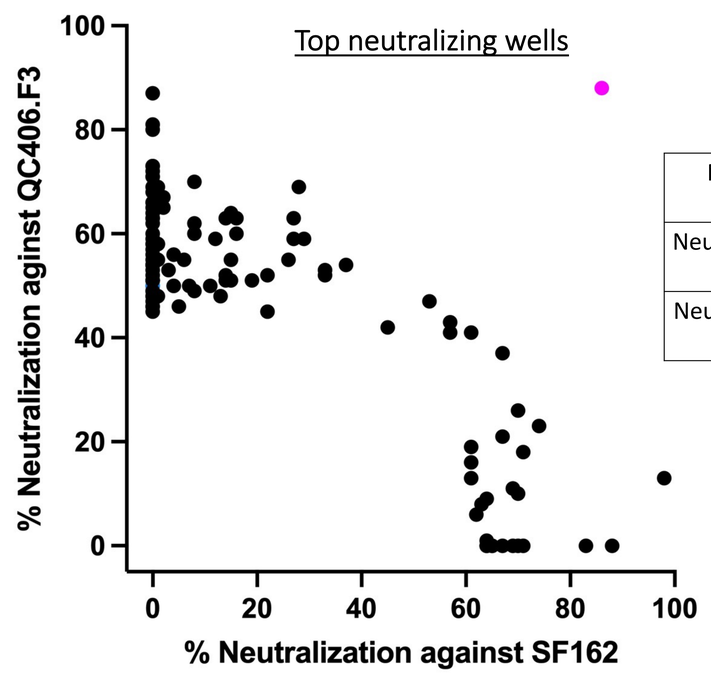


B cell culture supernatant screened against a clade B virus. Values indicate percent neutralization. Only depicting values above 30% neutralization.

B cell culture supernatant, harvested from the same B cell plate, was screened against a clade C virus. Values indicate percent neutralization. Only depicting values above 30% neutralization.



- B cell culture supernatant was screened for neutralization against an easily neutralized Clade B tier 1B isolate SF162 and a more neutralization-resistant clade C tier 2 isolate QC406.F3
- Selected wells demonstrating neutralization of one or both viruses. Based neutralization percent cutoff on previous screens and neutralization-sensitivity of HIV isolates
  - Goal is to isolate members of this individual's B cell repertoire without excluding any moderate but broadly neutralizing samples



Neutralizes QC406 & SF162 >80%: **1 Well**
Neutralizes QC406 >80%,or SF162 >80%: **5 wells**
Neutralizes SF162 >60% or QC406 > 45% **118 wells**

- Isolated RNA from 124 different B cell culture wells
- Of highest interest are the wells that display evidence of neutralization against both viruses (such as the B cell well indicated in pink) as these are the most likely to have a B cell that encodes an antibody with broad neutralizing activity
- We also wish to isolate RNA from wells that neutralize as least one virus as we want to characterize all antibodies that contribute to this individual's plasma breadth

Work-in-Progress

- 124 B cell culture wells demonstrated moderate to potent neutralizing activity against two distinct isolates of HIV in the microneutralization screen
- Heavy and light chain genes will be amplified and paired
- mAbs produced from paired chains will then be functionally screened for neutralizing activity and tested to see whether antibodies recapitulate QB850 plasma neutralizing activity
- Longer term, we will investigate isolated antibodies evolution & inferred lineage development via deep sequencing of longitudinal samples and Bayesian phylogenetics

Acknowledgements

We would like to thank the women enrolled in the Mombasa, Kenya Cohort. We thank Mackenzie Shipley and Zak Yaffe for guidance and assistance with the initial B cell sorting, culturing and harvest of relevant samples. This work was supported by NIH R01 AI138709 to JO.

"This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. (DGE-1762114 grant number for FR). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation."