Antigen Affinity Ceiling for Selection of Functional Mutations and HIV-1 Neutralization

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BACKGROUND

Study Purpose: To assess the range of affinity (kinetic rates) that is permissible for germinal center (GC) priming in a CD4 binding-site (bs) HIV-1 bnAb precursor (CH235 unmutated common ancestor, UCA) knock-in murine model.

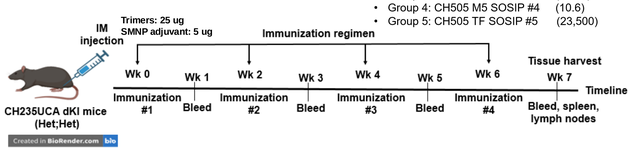
Hypothesis: A prime immunogen with the optimal affinity will favor germinal center (GC) selection of functional mutations and induction of autologous neutralization.

- CH235 (Vh1-46 CD4-bs) bnAb lineage evolved to 90% neutralization breadth, with no indels and normal CDR3 lengths (Gao et al., Cell, 2014 158(3):481-91 ; Bonsignori et al., Cell, 2016 165(2):449-63).
- CH235UCA binds with higher affinity to CH505TF trimers with select mutations (N279K, G458Y) and can neutralize such mutant pseudoviruses (LaBranche et al., PLoS Pathogens, 2019 15(9):e1008026).
- Critical improbable mutations acquired early in CH235 development (include K19T, shared among VH1-46 bnAbs) have been identified (Wiehe et al., Cell Host & Microbe, 2018 23(6):759-765.e6; Saunders et al., Science, 2019 366(6470):eaay7199).
- CH235UCA knock-in (KI) mice is a relevant model to test immunogenicity, particularly the selection of functional somatic mutations required for neutralization (Saunders et al., Science, 2019 366(6470):eaay7199).

METHODS

- Surface Plasmon Resonance: To measure the affinity and kinetic rates of CH505M5 transmitted/founder (TF) trimers to CH235UCA Fab.
- Calcium flux assay: To measure B cell activation.
- ELISA: Mouse sera antibody titer.
- TZM-bl assay: Pseudovirus neutralization.
- Immunophenotyping: Lymph nodes B cell phenotyping by flow cytometry.
- Next-generation sequencing (NGS): To identify functional mutations.

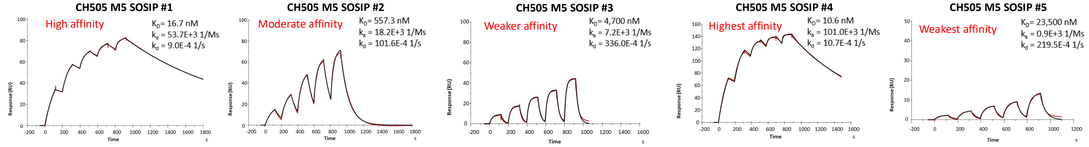
Experimental Groups: Affinities K_D (nM)
- Group 1: CH505 M5 SOSIP #1 (16.7)
- Group 2: CH505 M5 SOSIP #2 (557.3)
- Group 3: CH505 M5 SOSIP #3 (4,700)
- Group 4: CH505 M5 SOSIP #4 (10.6)
- Group 5: CH505 TF SOSIP #5 (23,500)



A lower and an upper affinity threshold is defined by antigen binding association and dissociation rates respectively, that set boundaries for selection of key functional mutations and the induction of higher titers of autologous neutralizing antibodies that target a CD4 mimic bnAb epitope.

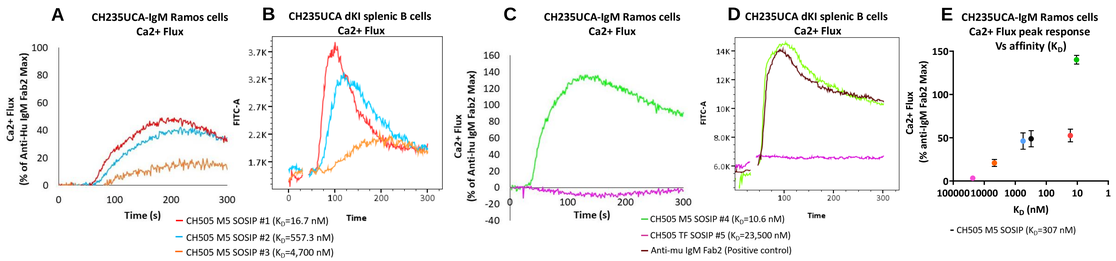
RESULTS

1. CH505M5/TF SOSIP trimers bind with varying affinities to CH235UCA Fab



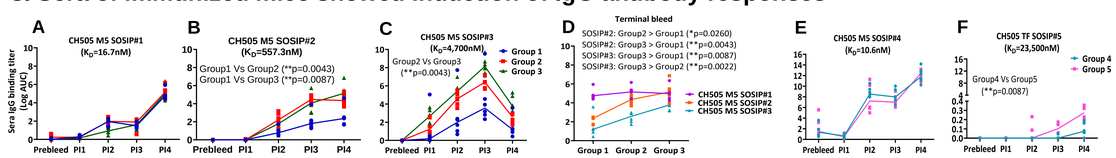
SPR binding of CH235UCA Fab to SOSIP trimers: SPR binding profiles of CH235UCA Fab to CH505 M5 SOSIP/293F and CH505 TF SOSIP/GnTI- trimers along with affinity measurements. CH235UCA Fab was injected using the single cycle kinetics injection (50 nM-3000 nM) over CH505 M5/TF SOSIP immobilized via biotin-streptavidin interaction. Trimers are ranked by affinity marked in red.

2. BCR signaling strength increased with increasing trimer affinities



Calcium (Ca2+) flux responses of CH235UCA-IgM expressing Ramos cell line and primary B cells from CH235UCA KI mice: (A and C) CH235UCA IgM Ramos cells were treated with SOSIPs (100 nM) and with the FLIPR Calcium 6 dye and monitored for 5 min using the Flexstation3 plate reader. **(B and D)** CH235UCA dKI (double KI, Vh+Vl) splenic B cells were treated with SOSIPs (50-100 ug/mL) or anti-mu IgM Fab2 positive control (50 ug/mL). Baseline response was collected for 30 sec and data was acquired for 5 min on a flow cytometer. **(E)** The plot represents Ca2+ flux peak response in Ramos cells versus affinity (K_D).

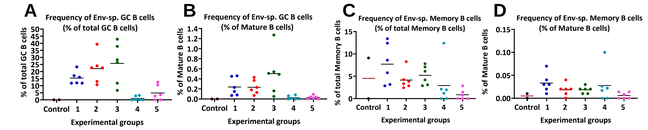
3. Sera of immunized mice showed induction of IgG antibody responses



Antibody IgG binding titers in sera samples collected from immunized CH235UCA KI mice: Sera IgG binding titers over time to a panel of **(A-E)** 293F cell grown CH505 M5 trimers **(F)** GnTI- cell grown CH505 TF trimer. The Y-axis represents antibody titer as the area under the $\log_{10}$ transformed concentration curve (logAUC). The lines connecting the groups are the median titer values for each group. Area under the curve was used for comparison between groups (Exact Wilcoxon test; *p<0.05, **p<0.01, ***p<0.001). PI: post immunization.

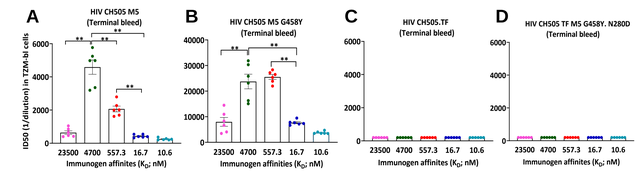
RESULTS

4. CH505 M5 trimers induced similar frequencies of Env-specific IgG+ GC and memory B cells in each group immunized with trimers of affinities ranging from 4,700 to 16.7 nM



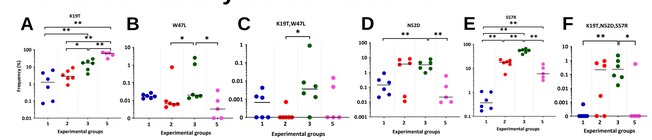
Lymph nodes B cell phenotypic analysis in immunized CH235UCA KI mice: Frequency of antigen-specific GC B cells as a % of **(A)** total GC B cells (Groups 1, 2, 3 > 4 p=0.0022; Groups 1, 2, 3 > 5 p=0.0130, 0.0043, 0.0152, respectively) **(B)** mature B cells (Group 1 > 4 and 5 p=0.0087; Group 2 > 4 p=0.0043; Groups 2 and 3 > 5 p=0.0043; Group 3 > 4 p=0.0087). Frequency of Env-specific memory B cells as a % of **(C)** total memory B cells (Group 1 > 4 and 5 p=0.0281, 0.0043, respectively; Groups 2 and 3 > 5 p=0.0065, 0.0022, respectively) **(D)** mature B cells (Groups 1, 2, 3 > Group 5 p=0.0087, 0.0325, 0.0216, respectively) (Exact Wilcoxon test). The flow cytometric B cell gating strategy for GC B cells used GL7, CD95, IgG1_2_3, and IgD markers whereas for memory B cells used CD23 and IgG1_2_3 markers along with antigen specific CH505 M5 G458Y GnTI- gp120 tetrameric hooks.

5. CH505 SOSIP trimers within a narrow range of affinities induced higher neutralization titers



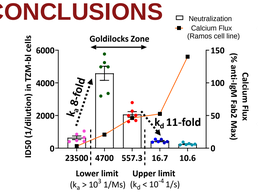
Pseudovirus neutralization titers of immunized CH235UCA KI mice sera: (A) Neutralization titers (ID_{50}) of CH505 M5 pseudovirus (tier2). The weaker affinity CH505 M5 SOSIP (Group 3, K_D=4,700nM) induced substantially high neutralization titers. **(B)** Neutralization titers (ID_{50}) of CH505 M5 G458Y pseudovirus. Relatively weaker neutralization levels were observed in each group with the moderate (Group 2, K_D=557.3nM) and the weaker (Group 3, K_D=4,700nM)-affinity CH505 M5 SOSIPs inducing significantly highest levels. **(C)** No neutralization activities were observed to CH505.TF pseudovirus. **(D)** Undetectable neutralization towards N280D mutated CH505 M5 G458Y pseudoviruses indicate that the induced sera antibodies were specific to the CD4-bs epitopes. (Saunders et al., Science, 2019 366(6470):eaay7199). Exact Wilcoxon test (**p<0.01).

6. GC selection of functional mutations favors moderate and weaker affinity BCR interactions



Splenic GC selection of functional mutations by CH505 M5/TF SOSIP trimer immunization: (A) NGS analysis of heavy chain variable regions (V_H) showed linear relationship between decreasing affinity and higher selection of functional improbable K19T mutation. The median frequency of **(B)** improbable V_HW47L mutation **(C)** combined V_HK19T+W47L mutation **(D)** functional probable V_HN52D mutation **(E)** functional probable V_HS57R mutation and (F) combined V_HK19T+N52D+S57R mutations were significantly higher in the weaker (Group 3, K_D=4,700nM)-affinity group. Mann Whitney test (*p<= 0.05, **p<= 0.01). Mutational frequency analysis were done for previously identified functional and improbable mutations (Wiehe et al., Cell Host & Microbe, 2018 23(6):759-765.e6; Saunders et al., Science, 2019 366(6470):eaay7199).

CONCLUSIONS



- A lower and an upper affinity threshold set boundaries for selection of key functional mutations and the induction of higher titers of autologous HIV-1 neutralizing antibodies.
- These studies show the importance of the optimal affinity/rates for GC priming with immunogen designs that target HIV bnAb epitopes.

ACKNOWLEDGEMENTS

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