Regulatory T-Cell Manipulation Is Limited by Anti-Antibody Responses in HIV-1 Env Immunized RMs

406

Shuqin Gu[1], Kan Luo[1], Tarra A. Von Holle[1], Thaddeus C. Gurley[1], Hilary Bouton-Verville[1], Xiaoying Shen[2], Georgia D. Tomaras[2], Barton F. Haynes[1], M. Anthony Moody[1]

[1] Duke Human Vaccine Institute, Duke University School of Medicine, Durham, NC 27710, USA;

[2] Duke University School of Medicine, Durham, NC 27710, USA.

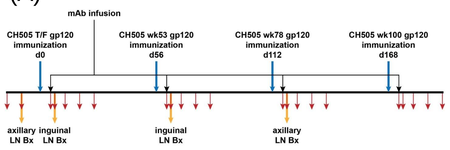
BACKGROUND

$CD25^+FoxP3^+CD4^+$ regulatory T (Treg) cells help mediate antigen tolerance in immune responses. We aimed to dissect the influence of anti-CD25 monoclonal antibody (mAb) on the germinal center response to immunization. We tested three mAb treatment arms: inhibiting $CD25^+$ Treg cells (Basiliximab), depleting that population (Anti-Tac), and a control (anti-influenza CH65). We immunized a cohort of rhesus macaques (RMs) with sequential gp120CH505 Envelopes (Envs) that mimicked the mutational pattern[1-3] observed during HIV-1 infection and tested for the development of HIV-1 broadly neutralizing antibodies (bnAbs).

METHODS

Nine RMs were randomly divided into three groups that received 1 mg per infusion of Basiliximab, Anti-Tac, or CH65. The vaccine regimen for this study is shown in Figure A. Plasma antibody responses were assayed by ELISA. Linear epitope mapping of plasma antibodies was performed by peptide microarray. Immune cell phenotypes were evaluated by flow cytometry. Single-cell PCR and sequencing were conducted to define the antibody spectrum of HIV-1 Env-specific memory B cells derived from lymph nodes. IgM anti-IgG in RMs was assayed by ELISA.

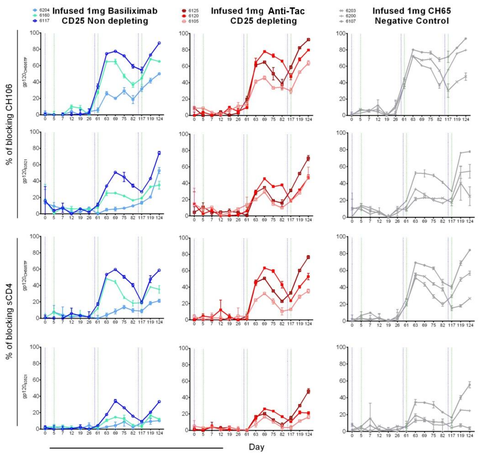
(A)



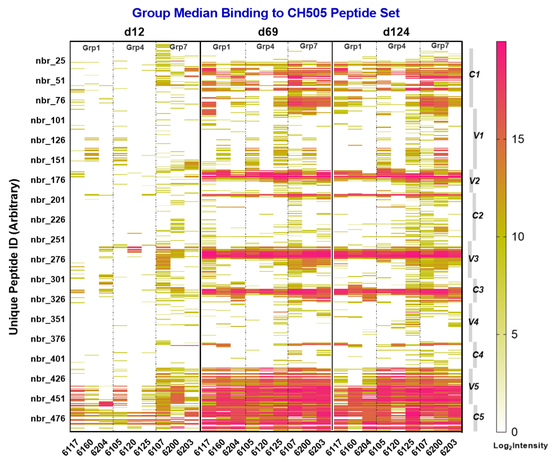
RESULTS

1. All RMs developed antibodies that blocked CH106 or sCD4 binding to $gp120_{CH505TF}$ and $gp120_{63521}$ (B). Binding to HIV-1 Env-specific linear epitopes trended lower after each immunization and infusion in RMs that received anti-CD25 treatment, compared to those received CH65 (C).

(B)



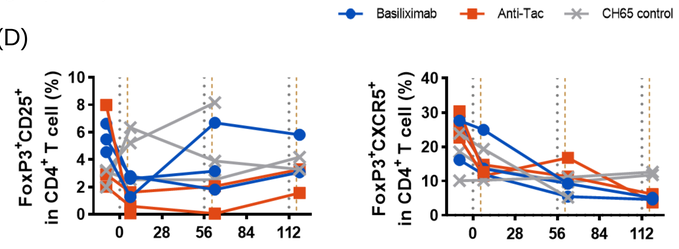
(C)



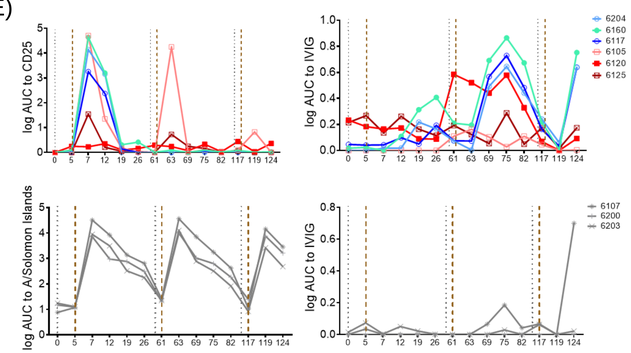
Treg perturbation strategies to induction of HIV-1 bnAbs is limited by anti-antibody responses in HIV-1-infected individuals.

2. Treg cell and CXCR5-expressing follicular Treg cell frequency was slightly decreased after the first anti-CD25 infusion but was similar after later infusions (D). Plasma anti-CD25 activity was detectable after the first infusion but limited after later infusions; CH65 infusion did not show the same pattern (E left panel). Anti-CD25 infused RMs developed rheumatoid factor after anti-CD25 mAb infusion that matched loss of anti-CD25 plasma activity. (E right panel).

(D)

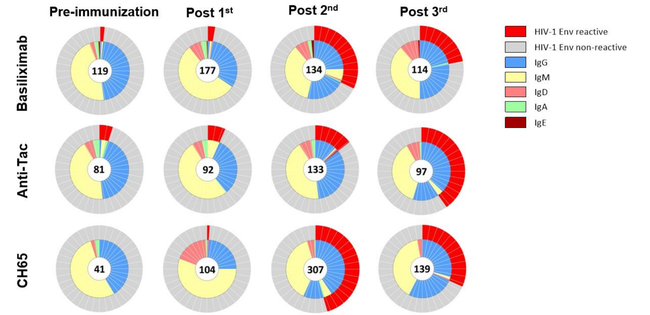


(E)



3. In pre- and post-1st immunization, only small numbers of isolated mAbs (0%~7.0%) were HIV-1 Env reactive. After the 2nd immunization, there was an expansion of HIV-1 antibodies in all groups, those in the Anti-Tac group were lower than that of Basiliximab and CH65 treated groups (14.3% vs. 32.1% and 45.6%). Compared to 15.6% of antibodies were clone involved in the control group, only 5% mAbs were clone related in both Basiliximab and Anti-Tac treated RMs.

(F)



(G)

	Basiliximab	Anti-Tac	CH65
Dose (mg)	1	1	1
Total mAbs	544	403	591
Total Clone No.	12	9	29
Total Involved Abs No. (% to total mabs)	28 (5.1%)	19 (4.7%)	92 (15.6%)
Env+ mAbs (% to total mAbs)	75 (13.8%)	68 (16.9%)	185 (31.3%)
Env+ Clone No.	2	2	24
Env+ involved mAs No. (% to Env+ mAbs)	4 (5.3%)	4 (5.9%)	72 (38.9%)

CONCLUSIONS

Transient Treg perturbation strategies to break immune tolerance and increase poly-reactive anti-Env antibodies in immunized RMs were limited by anti-antibody responses, indicating that immune manipulation strategies to drive the development of protective bnAbs may have off target effects.

ACKNOWLEDGMENTS

Support for this work was provided by the Center For HIV/AIDS Vaccine Immunology-Immunogen Discovery grant (CHAVI-ID; grant UM1 AI100645). This study was further funded by the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS grant UM1AI144371 for the Duke Consortia for HIV/AIDS Vaccine Development (B.F.H.).

Contact information: tony.moody@duke.edu

REFERENCES

1.Haynes BF, Kelsoe G, Harrison SC, Kepler TB; B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. Nat Biotechnol. 30, 423-433 (2012).
2.Liao HX, et al.; Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. Nature. 496, 469-476 (2013).
3.Gao F, et al.; Cooperation of B cell lineages in induction of HIV-1-broadly neutralizing antibodies. Cell. 158, 481-491 (2014).