Developing novel HIV-1 envelope proteins that efficiently engage the unmutated forms of VRC01-class antibodies

381

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BACKGROUND

VRC01-class antibodies are potent and broadly neutralizing antibodies (bnAbs) that protect animals from experimental S/HIV infection and have been shown to prevent HIV-1 acquisition in two phase 3 clinical trials. These antibodies target the CD4 binding site (CD4-BS) of the HIV-1 envelope glycoprotein (Env), but their predicted germline (gl) forms bind the Envs inefficiently. This is potentially why Env immunization has so far failed to elicit VRC01-class antibodies. We previously reported that specific modification of the clade C Env 426c lead to its binding by several glVRC01-class Abs.

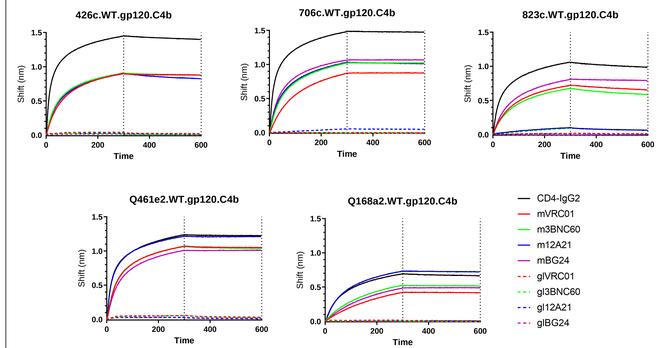


Figure 1: **The unmutated / germline forms of VRC01-class bnAbs do not bind rEnvs.** Mature (solid lines) and germline (dashed lines) VRC01-class mAb binding were evaluated against the indicated oligomeric Envs using Biolayer Interferometry assays. Black dotted lines indicate end of association and dissociation phases.

METHODS

Based on new structural information of Envs, we have now identified additional N-linked glycosylation site mutations that allow Envs from different clades:

- to bind glVRC01-class antibodies using Biolayer Interferometry assays
- to activate B cells engineered to express glVRC01-class B cell receptors *in vitro* using Ca^{2+} flux assay, and
- also activate the corresponding B cells *in vivo* when used as prime immunogens in knock-in mice expressing human VRC01-class B cell receptors

Figure 2: **Mature and germline forms of VRC01-class bnAbs bind differently to Modified.Core nanoparticles.** Black dotted lines indicate end of association and dissociation phases.

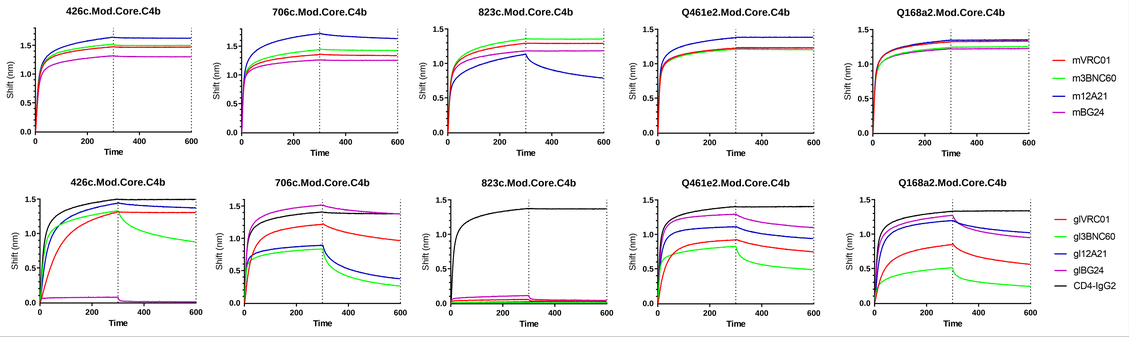
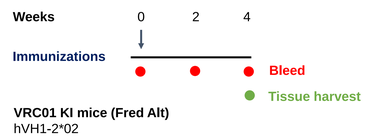


Figure 3: **B cells expressing different glVRC01-class BCRs show differential activation by Modified.Core nanoparticles.** Ca^{2+} flux analysis was carried out using Fluo-4 direct calcium indicator. Black arrows indicate time of antigenic stimulation.

Figure 4: **Evaluating the Modified.Core nanoparticles in VRC01 KI mice.** 3 to 4 mice/per group were immunized with indicated adjuvanted-Envs. Mice were bled at 2 weeks post-immunization with a terminal bleed and tissue harvest at 4 weeks.



Group	Immunogen + Adjuvant
GT1a	426c.Mod.Core.C4b + 3M-052-AF Alum
GT1b	Q168a2.Mod.Core.C4b + 3M-052-AF Alum
GT1d	Q461e2.Mod.Core.C4b + 3M-052-AF Alum
GT2a	706c.Mod.Core.C4b + 3M-052-AF Alum
GT2b	823c.Mod.Core.C4b + 3M-052-AF Alum

Figure 5: **Autologous Env- and VRC01 epitope- specific plasma antibody responses after prime are generated in each group.** ELISA was carried out for individual mice in each group against Envs as indicated in figure legend. Pre-bleed samples from all animals (as a pool) was used as an internal control.

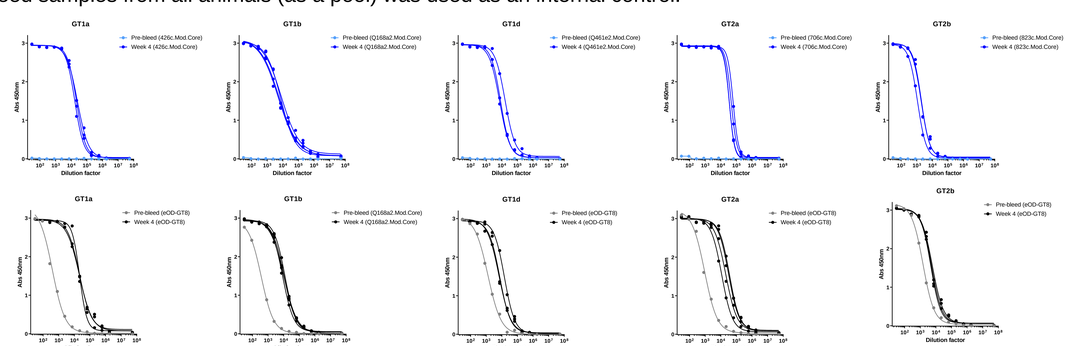
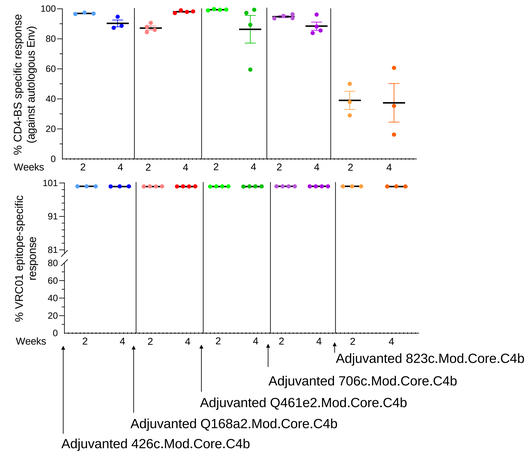


Figure 6: **CD4-BS-specific plasma antibody responses are elicited at weeks 2 and 4 after prime in each group.** Each colored circle indicates an individual mouse, and percent CD4-BS or VRC01-epitope specific response are calculated based on end-point titer values (error bars: s.e.m).



CONCLUSIONS

- Different Modified.Core nanoparticles bind inferred glVRC01-class Abs differently with 823c.Mod.Core.C4b showing negligible binding to all gl-Abs.
- They differently activate B cells expressing different glVRC01-class BCRs.
- Autologous and VRC01 epitope specific plasma IgG responses are induced after a single immunization in all vaccine groups.
- High proportion of the autologous plasma IgG responses are CD4-BS specific in the different vaccine groups (exception: 823c.Mod.Core.C4b).
- Our findings are central to efforts to engage a broad spectrum of VRC01-class precursors in clinical trials.

FUTURE DIRECTIONS

- Env specific B cell sorting and BCR sequencing for each vaccine group
- Ab generation and characterization
- Structural studies with a combination of Modified.Core nanoparticles and glVRC01-class Abs

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