Improved Immunogenicity of an HIV-1 VLP-Forming mRNA Vaccine

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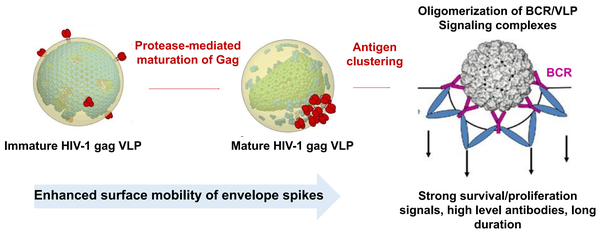
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BACKGROUND

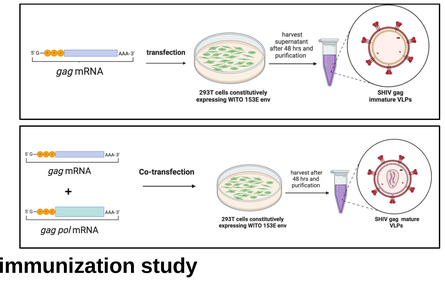
The HIV-1 envelope (Env) is a primary target for broadly neutralizing antibodies and a key focus for vaccine development. Effective engagement of the immune system requires optimal presentation of Env to B cells. We previously designed and pre-clinically tested a virus-like particle (VLP)-forming env-gag mRNA vaccine that elicited tier-2 neutralizing antibodies and protection against heterologous SHIV infection in rhesus macaques. Here, we further optimize this platform by inclusion of the viral-protease to process Gag and produce mature, rather than immature, VLPs. This study demonstrates that addition of a viral protease to process Gag into mature VLPs facilitate Env clustering on VLPs, improving B-cell activation and immune response which may impart enhanced effectiveness.

PROPOSED HYPOTHESIS



METHODS

***In-vitro* Expression of Immature and Mature SHIV-1 VLPs mRNA Formulation**



Schematic of immunization study



BALB/c

Mouse group	No. of mice	HIV-1 *env* mRNA	SIV *gag* mRNA	SIV *gag-pol* mRNA	*gag-pol:* *gag* mRNA ratio
1	8	2.5µg	-	-	-
2	8	2.5µg	2.5µg	-	-
3	8	2.5µg	2.5µg	0.5µg	1:5
4	8	2.5µg	2.5µg	0.25µg	1:10
5	8	2.5µg	2.5µg	0.125µg	1:20

RESULTS

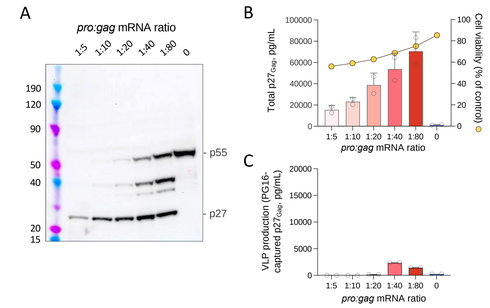


Fig. 1. Addition of mRNA expressing a retroviral protease results in efficient Gag processing but inefficient extracellular VLP production. (A) Western blot analysis of Gag p55 processing in stably Env-expressing HEK293T cells co-transfected with SIV gag mRNA and pro mRNA. (B) Quantification of total Gag p27 concentration in the culture supernatant of transfected cells. (C) Quantification of VLP associated Gag p27 captured using PG16 mAb from the culture supernatants

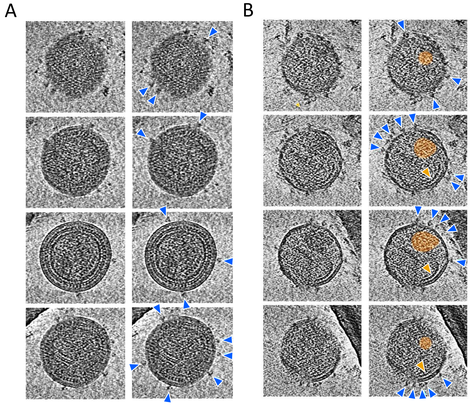


Fig. 3. Ultrastructural features of mRNA-expressed immature and mature HIV-1 VLPs. (A) Cryo-electron tomogram of an immature virus-like particle (B) mature virus-like particle. Serial computational sections through the 3-D tomogram shown from top to bottom. Left column raw tomogram, right column annotated to indicate Env (blue arrows) and Gag core (orange shaded) as well as a second partially formed core (orange arrow). Scale bar 100 nm.

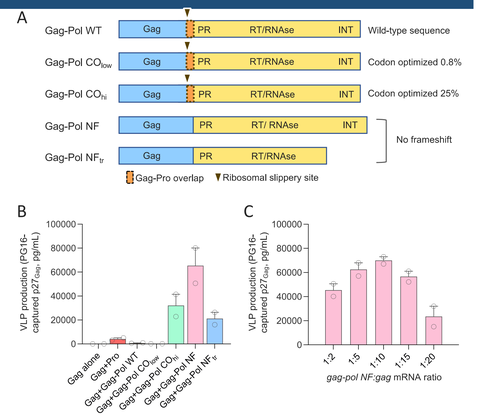


Fig. 2. Design and evaluation of different gag-pol mRNA constructs for expression of the SIV protease and production of mature VLPs. (A) Schematic representation of five different SIV gag-pol mRNA constructs (B) Quantification of extracellular VLPs produced by stably Env expressing HEK293T cells co-transfected with SIV gag mRNA and different gag-pol mRNA constructs or controls. (C) Quantification of VLP-associated Gag p27 captured from the culture supernatants of cells co-transfected with gag mRNA and decreasing amounts of gag-pol NF mRNA.

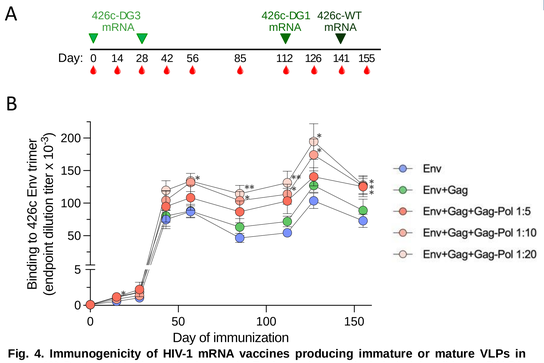


Fig. 4. Immunogenicity of HIV-1 mRNA vaccines producing immature or mature VLPs in mice. (A) Design of the immunization study. Each vaccine arm included eight wild-type BALB/c mice. Mice were immunized with LNP-formulated mRNA encoding HIV-1 426c Env and SIV mac239 Gag with or without Gag-Pol NF. (B) Induction of 426c trimer-binding antibodies in the 5 study arms over time, as assessed by ELISA. Statistical comparisons were made by Kruskal-Wallis test followed by post-hoc Dunn's correction for multiple comparisons.

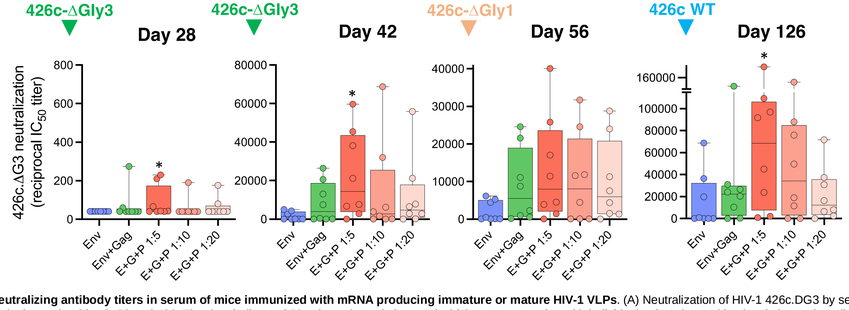


Fig. 5. Neutralizing antibody titers in serum of mice immunized with mRNA producing immature or mature HIV-1 VLPs. (A) Neutralization of HIV-1 426c.DG3 by sera from individual immunized mice at day 28, 42, 56 and 126. The data indicate IC50 values shown in box-and-whisker representation with individual points denoted by the circles and median values denoted by the horizontal line.

RESULTS

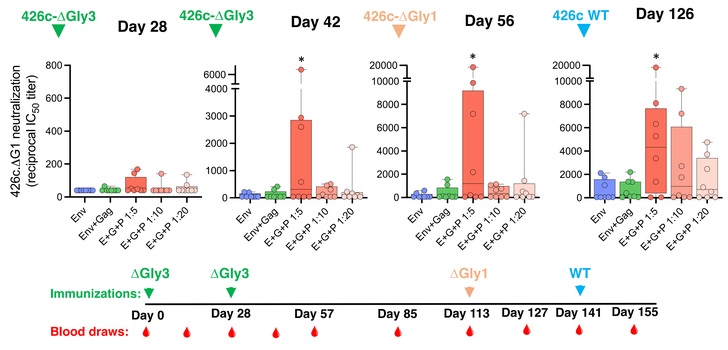


Fig. 6. Neutralizing antibody titers in serum of mice immunized with mRNA producing immature or mature HIV-1 VLPs. (A) Neutralization of HIV-1 426c.DG1 by sera from individual immunized mice at day 28, 42, 56 and 126. The data indicate IC50 values shown in box-and-whisker representation with individual points denoted by the circles and median values denoted by the horizontal line.

CONCLUSIONS

- Inclusion of the viral protease (*gag-pol*) in a VLP-forming HIV-1 mRNA vaccine for processing Gag results in the production of mature virus-like particles (VLPs) which display clusters of Env trimers on their surface.
- The optimized VLP-forming *env+gag+gag-pol* mRNA vaccine elicited higher titers of trimer-binding antibodies in mice compared to the protease-free vaccine producing immature VLPs
- The protease-supplemented mRNA vaccine was more efficient than *env-gag* mRNA in inducing neutralizing antibodies in mice

ACKNOWLEDGEMENTS

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