Cytoplasmic Tail Engineering of Stabilized mRNA Env Immunogens Enhances Neutralizing Response

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Background

Certain Env features, such as low cell surface expression and conformational lability, have hampered mRNA vaccine development for HIV. We previously found that ablation of Env endocytosis motifs and incorporation of a truncated HIV/SIV chimeric cytoplasmic tail (SIV.CT) increased surface expression on virions and cells transduced with vaccinia-vectored immunogens. Here, we incorporated this SIV.CT modification into mRNA immunogens stabilized to express prefusion trimers and measured the impact of CT engineering on cell surface expression and immunogenicity.

Methods

mRNAs encoding prefusion-stabilized versions of Subtype A, AE, C, and D HIV-1 Envs, including germline targeting Envs RC1 and 11MUTB, were produced. Cell surface protein expression from mRNA was assessed following transfection of 293F cells and staining with conformationally-dependent antibodies (*e.g.*, PGDM1400, 17b) using flow cytometry. To test immunogenicity, BALB/c mice were immunized intramuscularly at weeks 0 and 4 with 1 ug of mRNA-lipid nanoparticles encoding SIV.CT or gp160 variants of RC1 or 11MUTB. Serum drawn at weeks 2, 3, and 6 was evaluated using autologous antigen-specific ELISAs and neutralization assays.

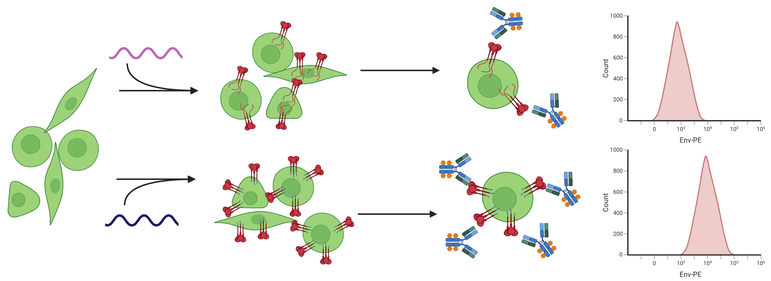


Fig. 1 Flow cytometry assessment of mRNA Env immunogens. Membrane-spanning Env immunogens encoded by mRNA are transfected to 293F cells. Transfected cells are stained with antibodies, binding of which can be quantified using flow cytometry. Indicated is an example in which two Envs with different length cytoplasmic tails have different levels of Env surface expression and the correlating change to fluorescence signal as measured with flow cytometry.

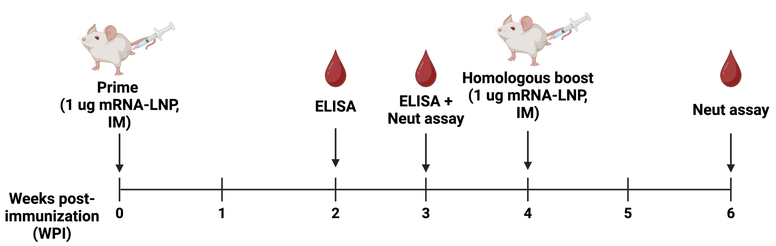


Fig. 2 Mouse immunization schematic. BALB/c mice were with 1 ug of mRNA-LNP vaccines intramuscularly. Boost was homologous. Mice were bled at three time points, weeks 2, 3, and 6, for assessment of the serum antibody response. Mouse serum was tested for autologous binding titers using ELISA and autologous neutralization using a TZM-bl assay.

Results

Strain	Subtype
BG505	A
11MUTB (BG505)	A
RC1 (BG505)	A
Q23	A
CAP256	C
Ce1176	C
CH505	C
16055	C
191859	D
C1080	CRF_01/AE
C4118	CRF_01/AE
CM244	CRF_01/AE

Table 1 HIV-1 strains selected for expression as mRNA-encoded immunogens. 10 strains with representation from subtypes A, C, D, and CRF_01/AE were selected. These include strains commonly employed to develop vaccines, germline targeting variants (RC1 + 11MUTB), and Envs that are sensitive V2-apex bnAbs to facilitate assessment of properly folded trimers in our flow-based assay.

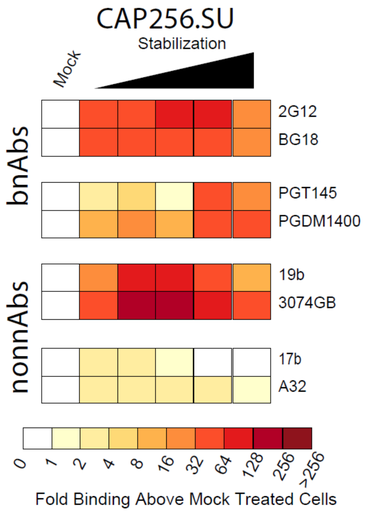


Fig. 3 Application of approaches used in protein immunogen development to generate prefusion-locked mRNA-encoded Env immunogens. Combinations of stabilization mutations confer V2-apex binding and reduce nonnAb binding in flow-based assay.

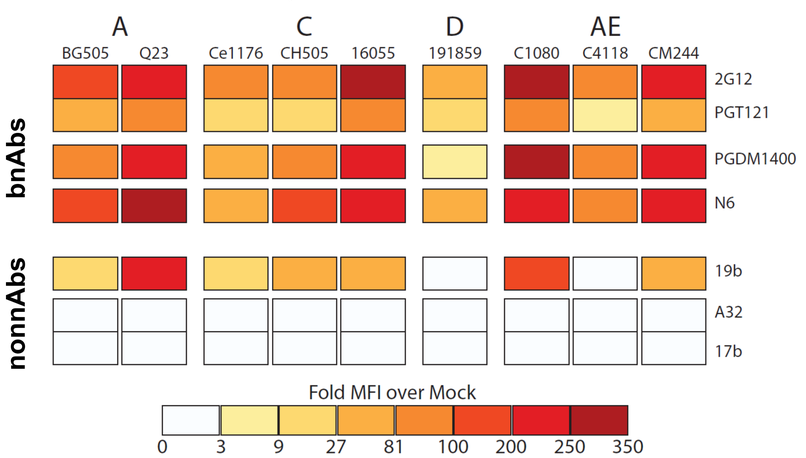


Fig. 4 Generation of genetically diverse Env mRNA immunogens. Flow cytometric analysis of nine mRNA-encoded HIV-1 immunogens reveals prefusion trimer expression. Minimal CD4i nonnAb binding was seen with high binding of bnAbs that target diverse epitopes. Note, Envs demonstrate a spectrum of IC50s for each antibody, which may impact binding. For instance, 191859.D has a PGDM1400 IC50 ~5ug/ml.

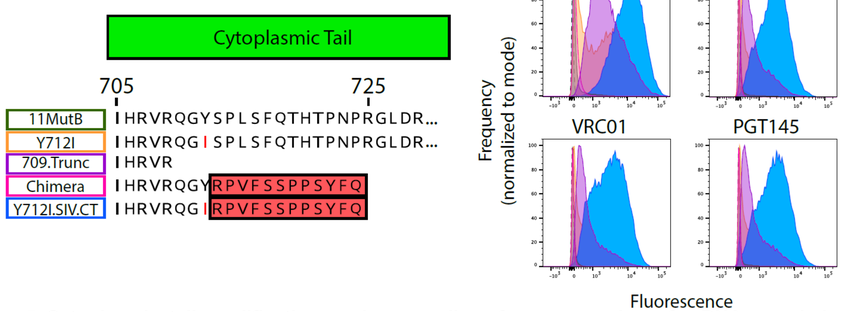


Fig. 5 Cytoplasmic tail modifications enhance cell surface expression of mRNA-encoded Env. Left: alignment of five cytoplasmic tail variants of 11MUTB were designed. These include variants with ablation of the Tyr-dependent endocytosis motif via Y712I, complete truncation of the cytoplasmic tail, or generation of a short, chimeric tail. Right: flow-based analysis of these 5 mRNAs.

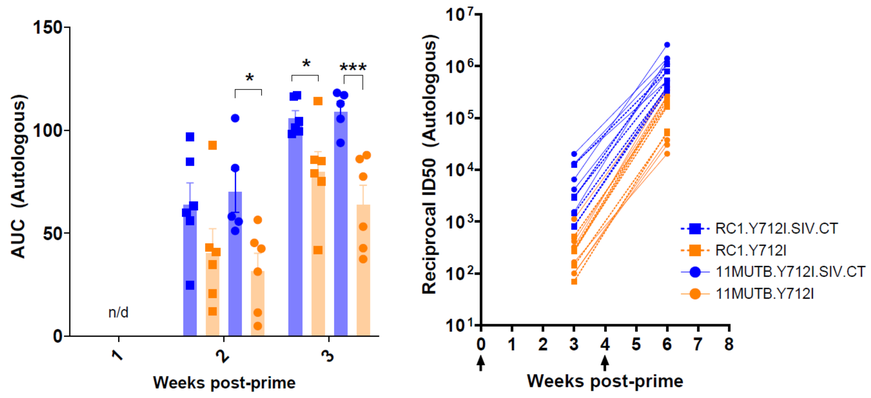


Fig. 6 Binding and neutralizing titers in BALB/c mice immunized with Y712I and Y712I.SIV.CT immunogens. Left: Area under the curve analysis of Env-specific ELISAs for sera collected at weeks 2 and 3 post-priming immunization. Right: Autologous neutralizing titers at weeks 3 and 6. Y712I.SIV.CT immunogens (blue) both demonstrate significantly higher binding and neutralizing antibody titers.

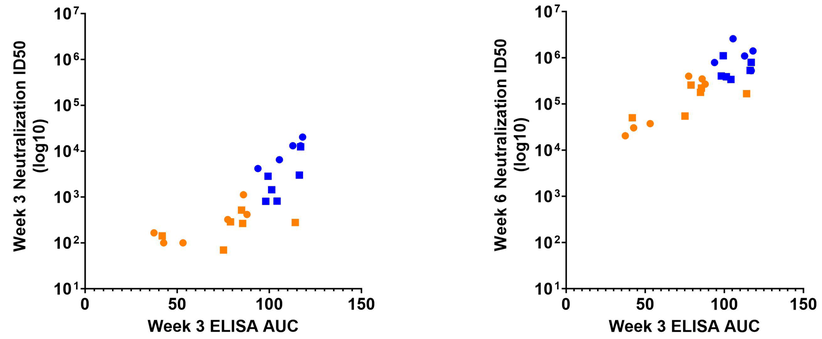


Fig. 7 Correlation of binding and autologous neutralization titers. Neutralization titers of week 3 (left) and week 6 (right) serum were compared to ELISA titers. Both measures of immunogenicity were directly correlated with highest neutralization titers seen among mice with the greatest binding titers. Additionally, mice that received immunogens with higher cell surface expression in 293F cells (Fig. 5, blue) were distinguishable from mice that received a lower expressing immunogen (Fig. 5, orange). Symbols are the same as Fig. 6.

Conclusions

Engineering of mRNA-encoded Env immunogens can:

-Generate prefusion-stabilized trimers

-Increase cell surface expression

-Increase immunogenicity

These immunogen design principles can be applied to preventative and therapeutic HIV vaccine development

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