mRNA vaccine candidates expressing stabilized Env trimers, some presented on HTLV-1 virus-like particles, elicit broad and potent neutralizing antibodies

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Background:
Human T-cell Lymphotropic Virus type 1 (HTLV-1) subtype C establishes lifelong infection of approximately 37% of people in some First Nations communities in Central Australia. Most people living with HTLV-1c develop effective virus-neutralizing antibody (nAb). Vaccines that can stimulate these preventive responses are needed. We developed mRNA–lipid nanoparticle (mRNA-LNP) vaccines encoding prefusion-stabilized HTLV-1 Env, presented as soluble trimers or Env tethered on the surface of VLPs, to elicit protective immune responses against HTLV-1.

Methods:
Mutations designed to stabilize Env pre-fusion trimer, reduce biofilm formation and enhance secretion of engineered Gag particles were tested and combined into lead candidates. mRNA-LNPs were produced from T7 *in vitro*-transcribed mRNA formulated with GenVoy lipids on a PNI NanoAssemblr. mRNAs encoding HTLV-1 Gag VLP and wildtype or engineered Env were expressed singly or combined to generate soluble Env trimers or Env-coated VLP. Optimized mRNA-LNP vaccines were injected intramuscularly into groups of ten C57BL/6J mice with 4 doses and sera were used for anti-Env pseudovirus neutralization.

Results:
Immunoblot analysis confirmed HTLV-1c mRNA expression of soluble, cell-membrane-bound, and Gag VLP-tethered stabilized Env trimers with mutation-dependent expression efficiencies. Transmission electron microscopy confirmed VLP formation with differing Env packaging and release. Following mRNA-LNP vaccination, mouse sera showed comparable anti-Env IgG levels across immunization groups by ELISA, but membrane-tethered Env on HTLV-1 Gag VLPs elicited higher nAb response after fewer doses. The best designs elicited robust Env nAb responses after the 3rd dose, and VLP versions expanded immunity to include Gag; both types showed potential as HTLV-1 vaccine candidates.

Conclusion:
Silent HTLV-1 endemicity is a global health concern and an effective nAb-powered vaccine is needed to prevent transmission. This study establishes an mRNA-LNP approach for HTLV-1 Env, confirms suitable antigen expression from mRNA *in vitro*

and shows in mice that select mutation-optimized constructs warrant further HTLV-1 vaccine development.

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