A Short Tale: Cytoplasmic Tail Modifications of an HIV Env Immunogen to Enhance Cell Surface Expression and Vaccine Immunogenicity

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The HIV Envelope protein (Env) is a central target in HIV vaccine design, yet intrinsic features limit antigen availability, motivating immunogen engineering strategies. During natural infection, Env surface expression is tightly regulated by internalization motifs (GYxxΦ) within its long cytoplasmic tail (CT). While advantageous for viral replication, this mechanism limits Env availability for immune recognition in vaccine contexts. Our lab has previously found that disrupting this motif (ΔGY/Y712I) and incorporation of a truncated HIV/SIV chimeric CT increased surface Env and IgG titers following immunization (SIV.CT). Additional infection-based studies have identified mutations (S727P and R722G) in the CT that further enhance Env surface expression. Here we introduced those viral mutations into our SIV.CT mRNA-encoded Env immunogens containing a disrupted GYxxΦ motif (ΔGY/Y712I). HEK 293F cells were transfected with mRNA constructs and cell surface expression was analyzed via flow cytometry using a panel of conformationally dependent monoclonal antibodies. mRNA constructs were then formulated into lipid nanoparticles and used to vaccinate BALB/c mice intramuscularly at weeks 0 and 4 at a 0.8μg dose. Serum drawn at weeks 3 and 6 post prime were evaluated using autologous ELISA assays. All CT modifications resulted in a ~1 log increase in Env cell surface expression relative to the wild-type (WT) Env (measured via mean fluorescence intensity), with S727P-containing variants consistently achieving the highest expression independent of the GYxxΦ deletion strategy (Y712I, S727P vs. Y712I and ΔGY, Y712I vs. ΔGY Fold over Mock: 404 vs. 340 and 380 vs. 285). Following homologous boost, S727P-containing variants elicited ~ 2.5-3-fold higher antibody binding compared to WT at 6 weeks post-prime (Y712I/S727P vs. WT geomean AUC: 217,749 vs.86,932, geomean end point titer: 375,250 vs. 130,190). These findings identify S727P-containing CT variants as optimal candidates for enhancing Env surface expression without sacrificing conformation integrity.