Ablating endocytic motifs within Env mRNA vaccines enhances cell surface expression and immunogenicity

Perez Devia, Natalia[1], Brock, Breezy N.[1], Ding, Wenge[1], Albertus, Amie[1], Skelly, Ashwin N.[1], Xiao, Yuhong[1], Liu, Weimin[1], Ni, Houping[1], Bjorkman, Pamela J.[3], Haynes, Barton F.[2], Shaw, George M.[1], Hoxie, James[1], Wagh, Kshitij[2], Hahn, Beatrice H.[1], Weissman, Drew[1], Kreider, Edward F.[1]

[1]University of Pennsylvania; [2]Duke University; [3]Caltech

HIV Env has proven a challenging immunogen for mRNA vaccine development due to its low cell surface expression and conformational lability. Animals vaccinated with mRNA-lipid nanoparticle (LNP) vaccines encoding wildtype Envs infrequently generate neutralizing antibodies. This is in part due to a highly conserved Tyr-based trafficking signal (GYXXΦ) within the Env cytoplasmic tail (CT) that mediates clathrin-dependent endocytosis of Env and contributes to immune evasion during infection. Previous work identified a truncated SIV Env variant with ablation of this motif that, in the setting of live virus, increased Env content on the surface of infected cells. Here, we incorporated this truncated SIV.CT and endocytosis motif ablation individually and in combination in prefusion-stabilized mRNA Env immunogens to assess their roles on cell surface expression and immunogenicity.

Nucleoside modified mRNAs encoding prefusion-stabilized germline targeting Env (11MUTB.MD39.SOS.DS.IP.PP) were designed with various CT modifications (WT, Y712I, SIV.CT, or Y712I.SIV.CT). mRNAs were transfected into HEK293F to assess cell surface expression using a conformationally-dependent panel of broadly neutralizing antibodies (bNAbs) (e.g., PGDM1400, 2G12) or non-neutralizing antibodies (e.g., 17b) using flow cytometry. BALB/c mice were immunized intramuscularly at weeks 0 and 4 with 0.1-1.0 ug of mRNA-LNP. Sera were drawn at 3 and 6 weeks post-prime for autologous neutralization assays and ipsilateral lymph nodes were collected to assess prime germinal center B cell responses.

Constructs containing the combination of both Y712I.SIV.CT demonstrated increased cell surface expression of prefusion trimers, as demonstrated by increased 2G12 (18-fold), N6 (10-fold), and PGDM1400 (8-fold) signal over Env with a wildtype CT. Regardless of level, all constructs demonstrated low 17b binding, indicating that CT modifications did not induce conformational changes in the ectodomain. Sera from mice vaccinated with the Y712I.SIV.CT variant demonstrated greater 11MUTBSOSIP binding titers at week 2 (EPT 663 vs 108) and week 3 (EPT 5457 vs 688) post-prime when compared to wildtype CT. These included 10-28-fold greater SHIV.11MUTB neutralizing titers 3 weeks post-prime (geomean reciprocal ID50: 7,246 vs. 251) that remained 3.7-11.7-fold higher at week 2 post-boost. Neutralizing and binding titers elicited in mice positively correlated with one another. Lymph nodes sampled from Y712I.SIV.CT immunized mice showed a trend toward greater number of total germinal center B cells, even at 0.3ug and 0.1ug doses. Interestingly, tails harboring either Y712I or the SIV.CT yielded lower cell surface expression and neutralizing titers than Y712I.SIV.CT, suggesting that both are required for maximal enhancement of immunogenicity. Env CT variants that demonstrate increased Env virion content can be incorporated into mRNA immunogens to yield higher cell surface expression and immunogenicity. Importantly, when used in combination with prefusion-stabilizing mutations, these CT changes maintain prefusion antigenicity, which is needed to elicit bNAbs.