Enhancing HIV-1 Vaccine Efficacy and Manufacturing Through CHO Cell CRISPR Engineering

Cell & Gene Therapy

Keywords: *Chinese hamster ovary (CHO) cells, CRISPR, genome engineering, cell line development, human immunodeficiency virus (HIV), vaccine, protein production, MaxCyte STX®, electroporation*

Background

The RV144 HIV vaccine trial, the first trial to demonstrate that vaccination could confer HIV protection, reported a modest 31% efficacy. gp120, a major component of the RV144 trial, was produced in Chinese Hamster Ovary (CHO) cells and lacked N-linked glycosylation sites critical for binding of anti-HIV broadly neutralizing antibodies (bN-mAbs). Researchers believe, at least in part, that the lack of N-linked glycosylation may play a role in the poor efficacy.

Many epitopes bound by bN-mAbs contain oligomannose terminal glycans — early intermediates in the N-linked glycosylation pathway.[1] Dr. Berman's group at University of California Santa Cruz demonstrated using MaxCyte electroporation that recombinant gp120 (rgp120) production using a HEK 293 cell line deficient in the N-acetylglucosaminyltransferase I enzyme (GnTI-293; ATCC CRL-3022) resulted in HIV epitopes containing mainly mannose-5 terminal glycans.[2] The presence of these glycan moieties improved binding of three major bN-mAb families suggesting use of these rgp120 during vaccination may result in improved efficacy. GnTI$^-$ 293 cells, however, are not suitable for large, clinical-scale bioproduction hindering migration to *in vivo* efficacy studies.

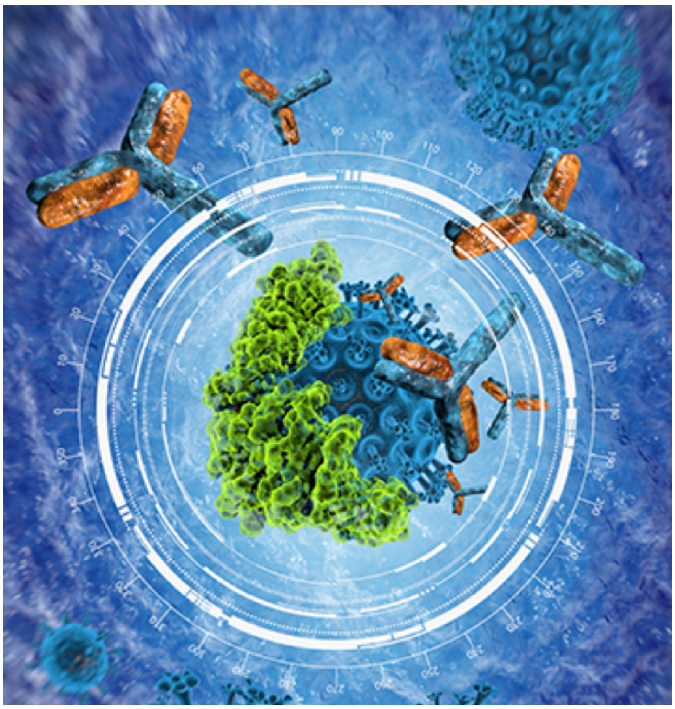
CHO cells continue to be the gold standard for biotherapeutic development, however, the complexity and heterogeneity of protein glycosylation can pose manufacturing and/or immunogenicity challenges. Creation of a CHO cell line deficient in alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase (MGAT1) would produce proteins primarily containing oligomannose glycans and could greatly improve HIV gp120 production, purification and potentially vaccine efficacy.

Aim

Generate a CHO MGAT1$^-$ cell line via CRISPR gene knockout that maintains growth properties suitable for use in protein manufacturing. In addition, transiently produce rgp120 using the engineered CHO MGAT1$^-$ cell line and characterize protein glycosylation patterns and bN-mAb binding.

CHO Electroporation

- CHO-S or CHO MGAT1$^-$ cells were resuspended in MaxCyte electroporation buffer at 2 x 10^8 cells/mL.
- For CHO MGAT1$^-$ construction, CRISPR/Cas9 exonuclease with guide sequence plasmid was added to resuspended CHO-S at a final concentration of 300 µg/mL.
- For transient expression of HIV gp120, an expression plasmid encoding the gp120 from the A244 HIV strain were added to CHO MGAT1$^-$ cells.



- Post electroporation, cells were seeded at 4 x 10^6 cells/mL in OPTI-CHO media and cultured in 125-mL Erlenmeyer shake flasks.
- For gp120 production, cultures were supplemented with 1 mM sodium butyrate 24 hours post electroporation and the temperature lowered to 32°C.

Full methods for CHO MGAT1$^-$ screening and selection, as well as glycosylation analysis and binding assays are detailed in PLoS Biol, 16(8): e2005817, 2018.

Results

CRISPR Delivery & Creation of CHO MGAT1$^-$ Cell Line

CHO-S cells were electroporated with a plasmid encoding CRISPR/cas9, tracrRNA, and a complete guide RNA targeting the MGAT1 enzyme. Clones were initially screened for staining with fluorescein-labeled Galanthus nivalis lectin (GNA), a lectin that recognizes glycans with terminal mannose but not complex, sialic acid-containing glycans. As anticipated, the parental CHO-S cell line did not bind GNA. The four GNA-binding clones with the fastest growth rates were transiently transfected with a plasmid encoding A244-rgp120. Secreted rgp120 was purified and analyzed for overall titer and binding to the glycan-dependent bN-mAb PG9.

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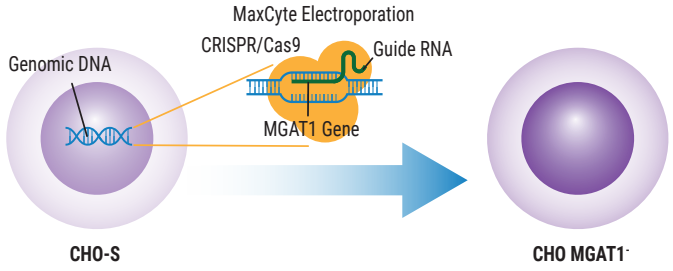
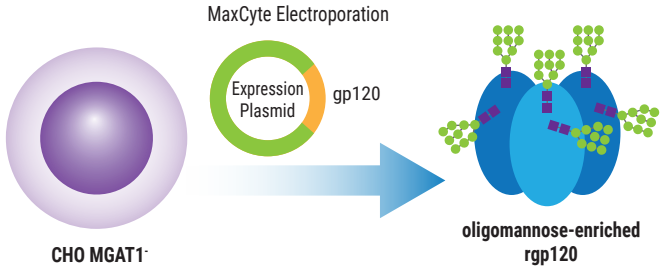
CHO-S Cell Line Engineering



Transient Expression of rgp120



The selected CHO MGAT1- cell line had rgp120 yields comparable to the parental CHO-S line, as well as similar doubling times and the ability to grow at high density. These characteristics were far superior to the yield, growth time, and cell density restrictions of the GnTI- 293 HEK cell line used in previous studies.[3] Sequence confirmation of the selected clone was performed to ensure disruption of the MGAT1 gene.

Identification of MGAT1- CHO Cells via GNA Binding of Oligomannose

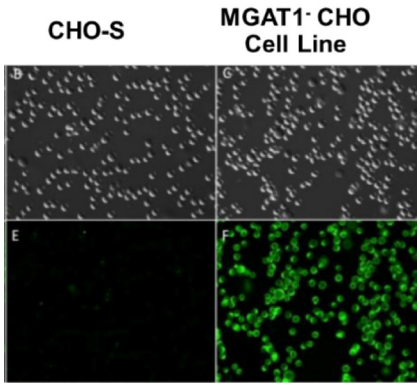


Figure 1: *CHO-S parental cells or those electroporated with MGAT-targeting CRISPR machinery were incubated with fluorescein-labeled GNA to identify cells with surface proteins containing terminal mannose residues.*

Transient Expression of rgp120

A224-rgp120 transiently produced using the selected MGAT1- CHO cell line or parental CHO-S cells was characterized by MALDI-TOF mass spectroscopy to detail glycan composition. MGAT1-deficiency resulted in rgp120 containing >99% oligomannose glycans compared to only 25% when produced in CHO-S cells.

The homogeneity of glycosylation resulting from MGAT1-deficiency also positively impacted purification. The rgp120 in the RV144 HIV clinical trial showed extensive net charge heterogeneity that required immuno-affinity chromatography for purification greatly reducing yield and increasing manufacturing complexity. rgp120 produced by MGAT1-deficient cells was secreted at high titers with homogeneous glycosylation allowing for purification using more conventional chromatography.

MGAT1-deficient CHO Cells Produce rgp120 Containing >99% Oligomannose Glycans

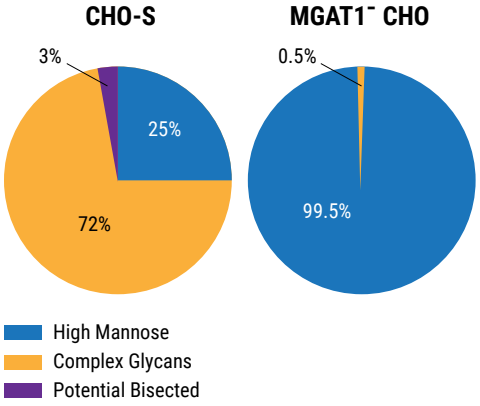


Figure 2: *Purified rgp120 from the selected CHO MGAT1- cell line or the parental CHO-S cell line were analyzed via MALDI-TOF mass spectroscopy.*

MGAT1-deficiency Produces rgp120 With Improved Binding to Multiple bN-mAbs

Binding of rgp120 produced in CHO-S or MGAT1- CHO cells to a panel of bN-mAbs was assessed via fluorescence immunoassay.[3] All but a single antibody had modest to significant improvements in binding to rgp120 produced in the MGAT-deficient cells. These results suggest that altering glycosylation while leaving the amino acid sequence unchanged can positively impact bN-mAb binding. Examination of the effects of oligomannose engineering on *in vivo* immunogenicity and vaccine efficacy are critical next steps.

Conclusion

MaxCyte's high performance cell engineering technology delivered the CRISPR/cas9 machinery that resulted in levels of efficiency and CHO-S cell viability that enabled the rapid generation of an MGAT1-deficient CHO cell line via precision CRISPR gene disruption. The established

Improved Binding of V1/V2 and V3 Domain bN-mAbs to rgp120 Produced in MGAT1-deficient CHO Cells

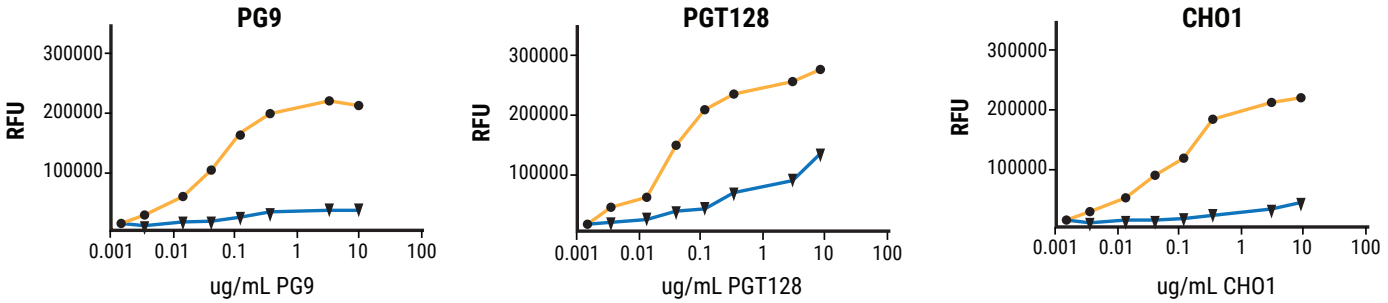


Figure 3: A224-rgp120 purified from CHO-S or MGAT1- CHO cells were coated onto microtiter plates and binding of a panel antibodies with broadly neutralizing capacity examined via FIA.

MGAT1- CHO cell line maintained robust growth in serum-free medium, was able to grow at high cell densities in suspension and produced rgp120 at high titers upon transient transfection. rgp120 produced using the newly engineered MGAT1- CHO cells gave rise to the desired early oligomannose glycans which in turn enhanced the binding of key bN-mAbs. This cell line promises to be a critical piece in the clinical translation of more efficacious HIV vaccines.

References

1. High-mannose Glycan-dependent Epitopes Are Fequently Targeted in Boad Neutralizing Antibody Responses during HIV-1 Infection. (2012) J Virol, 86(4):2153-2164.

2. Glycan Modification to the gp120 Immunogens Used in the RV144 Vaccine Trial Improve Binding to Broadly Neutralizing Antibodies. (2018) PLoS ONE, 13(4):e0196370.

3. CRISPR/Cas9 Gene Editing for the Creation of an MGAT1-deficienct CHO Cell Line to Control HIV-1 Vaccine Glycosylation. (2018) PLoS Biol, 16(8): e2005817.

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