Alter: A Genetic Approach to Optimizing the Antigenicity of HIV-1 Envelope

OVERVIEW

Over the past decade, a number of novel neutralizing Abs (nAbs) have been defined that target the V2 and neighboring loops that are highly exposed and may contribute to viral neutralization due to their involvement in trimerization. Many of these novel Abs show unique antigen-binding specificities, involving glycan-dependent specificities. Moreover, given that sugars consist of approximately half the mass of the HIV envelope, mounting evidence suggests that glycans interact with bNAbs against every target on the viral envelope. However, while previous efforts have manipulated individual glycans or groups of neighboring glycan to de-emphasize/emphasize particular Ab targets, to date, few immunogens have attempted to actively manipulate the overall HI envelope glycan composition, rather than target glycans. Thus, this project proposes to actively manipulate and exploit the overall composition, quality, and landscape of the HIV glycans to enhance protein antigenicity. Thus using cutting edge mass spectrometric glycomic analytical tools, genome screening technologies, and systems level machine learning tools, the HIV envelope glycome will be actively re-engineered to enhance the generation of higher quality vaccine anitgens to promote more effective humoal immunity.

RESEARCH OBJECTIVES

Phase 1:

1.) Objective identification of genetic modifications that improve gp120-1086 production

2.) Unbiased identification of genetic modifications that improve gp120-1086 antigenicity

3.) Cross-validation of “hits” in a CHO cell line for ultimate ENV-producer cell line development

Phase 2:

1.) Objective identification of gene modifiers that improve high quality SOSIP secretion

2.) Unbiased identification of gene modifiers that improve SOSIP antigenicitiy

3.) Identification of gene modifiers that drive enhanced In vivo immunogenicity

Grant at a Glance

Principal Investigator
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Grantee Institution
Massachusetts General Hospital, Boston, USA

Project Title
A Generic Approach to Optimizing the Antigenicity of HIV-1 Envelope Immunogens

OPPID
1097381

Grant Award
Up to $2.6 Million, awarded in November, 2013

Collaborating Institutions
- Complex Carbohydrate Research Center, University of Georgia Center
- University of Massachusetts Medical School

PROGRESS

This project brings together the genome-wide screening expertise of Dr. Abraham Brass (University of Massachusetts), the glycobiology expertise of Drs. Lance Wells and Mike Tiemeyer (Complex Carbohydrate Research Center), and the HIV immunology expertise of Dr. Alter (Ragon Institute/Harvard) to develop an approach to enhance the antigenicity of HIV ENV immunogens. In the first phase of the study, significant headway has been achieved in the following areas:

1.) Glycan composition, site occupancy, and site specific glycan analysis was performed on 94 individual HIV ENV proteins linked to antigenicity profiling by nAb and non-nAb binding. These reveal both the conservation as well as heterogeneity of glycosylation at specific glycan sites within the ENV protein. Machine learning algorithms used to interrogate glycan landscape profiles linked to antibody binding profiles across various proteins, clades, and tiers of viruses provides a novel predictive framework for the identification of glycan-dependent footprints of both previously characterized and uncharacterized monoclonals or polyclonal antibody populations. These proof of concept studies on recombinant gp120 monomers have led to the production of antigenically superior recombinant gp120s, able to evade non-nAb recognition, but bind potently to nAbs. These principles are now being applied to SOSIP molecules.

2.) The first genome-wide screen using 2 siRNA libraries (Ambion and Dharmacon) are now complete -identifying a number of hits that improved both ENV secretion and antigenicity.

- Remarkably, both ER and non-ER (transcriptional) hits were identified for enhancing protein production levels, suggesting a complex regulatory pathway that may be easily manipulated to improve therapeutic target protein production.

- No difference was observed in PGT121 and VRC01 antigenicity, but significant hits were observed for PGT128 antigenicity. A unique set of genes, involved in Golgi organization, vesicular transport, and metabolism were identified as enhancers of bnAb antigenicity.

However given the growing interest in native like trimers, confirmation/validation exercises were strategically transferred to a SOSIP screen. Within this validation screen, CRISPR/CAS9 will be used directly on CHO cells, and gene modifiers that improve the level of:

1.) high quality SOSIP (ratio),

2.) the antigenic profile of SOSIP, and

3.) the levels of high quality SOSIP will be defined.

Ultimately, collective, validated, converging hits will be computationally assembled and SOSIP proteins will be produced and tested in vivo for improved antigenicity and immunogenicity.