STRUCTURAL CHARACTERIZATION OF DNA-ENCODED HIV VACCINES INDUCED NEUTRALIZING ANTIBODY

00235

Ziyang Xu[1], Susanne Walker[2], Megan C. Wise[3], Neethu Chokkalingam[2], Mansi Purwar[2], Alan Moore[4], Jesper Pallesen[4], Daniel W. Kulp[2], David Weiner[2]

[1]University of Pennsylvania, Philadelphia, PA, United States, [2]Wistar Institute, Philadelphia, PA, United States, [3]Inovio Pharmaceuticals, Inc, Plymouth Meeting, PA, United States, [4]Indiana University, Bloomington, IN, United States

BACKGROUND

Rapid and large-scale deployment of COVID-19 mRNA vaccines highlights the potential utility of developing nucleic acid vaccines (such as RNA and DNA vaccines) against infectious diseases, including HIV-1. However, as compared to SARS-CoV-2, HIV-1 pose some unique challenges- induction of neutralizing antibodies (NAbs) against HIV-1 (frequently a correlate of protection) requires presentation of trimeric and highly conformational epitopes to the immune system, and whether nucleic acid vaccines can enable direct in vivo production of antigens that retain critical antigenic profile has not yet been elucidated. Additionally, it was previously reported that Tier 2 NAbs cannot be induced in mice due to a lack of antibody repertoire, and vaccine studies were suggested to be performed in larger mammals such as rabbits/ NHPs, inadvertently slowing down and increasing the costs of preclinical HIV-1 vaccine studies.

- DNA cassettes empower *in vivo* assembly of trimeric Env Antigens with identical antigenic profile as in vitro produced NLTs
- Autologous Tier 2 NAb responses can be achieved in murine models with DNA but not protein vaccinations
- Monoclonal Murine MAbs bind to C3/V5 epitope on HIV Env to mediate neutralization by occluding access to key CD4BS residues

METHODS

In our study, we used the Antigen Conformation Tracing In Vivo by ELISA (ACTIVE) assay developed in house to characterize antigenic profiles of vaccines produced in vivo (from transfected muscle tissues). We analyzed induced cellular responses, using stimulation with overlapping peptides followed by intracellular cytokine staining and IFN-g ELIspot assays. We analyzed induced humoral responses by using both binding ELISA assays and TZM-BL based neutralizing assays, and attempted to map induced NAb epitopes by engineering selectively mutated pseudovirus. We performed antigen-specific B-cell sorting, and used the 10x genomics pipeline to characterize antibody sequences of proliferating B-cell clones.

RESULTS

We confirmed that in vivo produced vaccines retained key trimeric conformational epitopes and glycan profiles. Compared to protein vaccination, DNA vaccination uniquely and strongly induced both TFH, CD4+, CD8+ T-cell responses, and Tier 2 NAbs mapped to a previously unreported Env C3/V5 epitope. 5 unique NAbs were isolated, and confirmed to bind to the epitope using a Cryo-EM structure of NAb-MD39 complex at 3.8Å resolution.

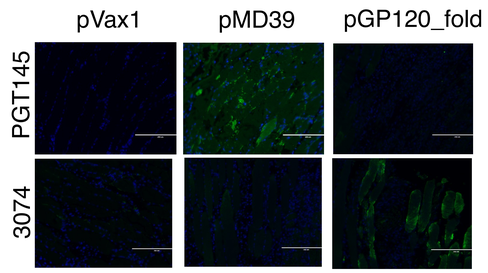
CONCLUSIONS

Our study confirmed that with appropriate vaccine delivery technology, murine models can be appropriately used for HIV-1 vaccine studies aimed at generating NAb responses. Additionally, detailed knowledge of epitope engagement of murine Fabs with HIV-Env can serve to inform structural design of next-generation vaccines to generate breadth in neutralization. Beyond potential functional immunity gains, DNA vaccines permit in vivo folding of structured antigens and provide significant cost and speed advantages for enabling rapid evaluation of new HIV vaccines.

ADDITIONAL KEY INFORMATION

The project is supported by IPCAVD U19 AI109646-04.

For further questions, please contact author at Ziyang.xu@Pennmedicine.upenn.edu.



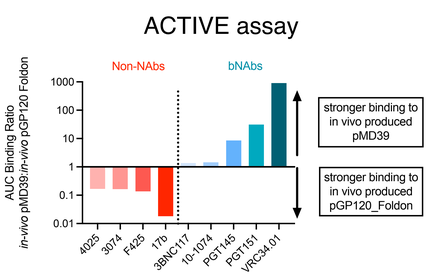


Exhibit 1: In vivo IF and ACTIVE Assays demonstrated DNA cassettes launched fully assembled trimeric HIV antigens in vivo

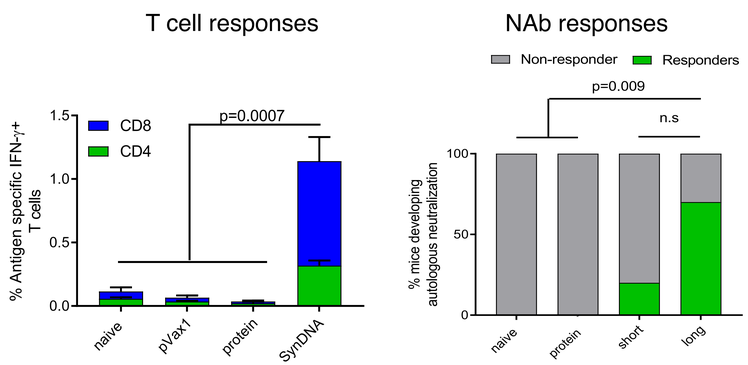


Exhibit 2: DNA vaccinations uniquely induced CD8+ T cell and neutralizing antibody responses in murine models as compared to protein vaccination

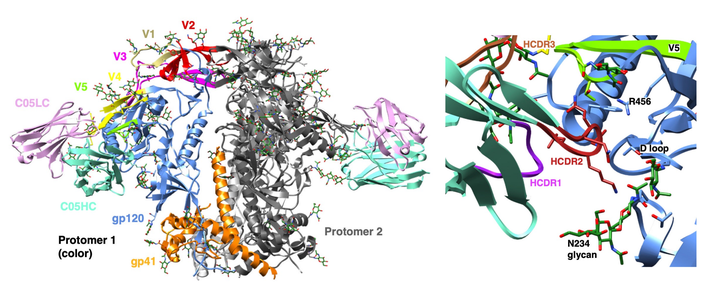


Exhibit 3: Mechanism of neutralization elucidated by CryoEM structure of Fab –Env complex

Perelman School of Medicine
University of Pennsylvania

THE WISTAR INSTITUTE