ADENOSINE DEAMINASE-1 ENHANCES HIV SPECIFIC RESPONSES TO A HIV TRIMERIC ENVELOPE DNA VACCINE

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1. Next Generation HIV Vaccines using Engineered Native Like Trimer HIV Envelope

Human immunodeficiency virus (HIV) remains a prominent global health threat for which no prophylactic vaccine is available. Extensive research efforts have resulted in immunogens, including SOSIP trimers, that closely mimic the native envelope (Env) glycoprotein conformation and consistently induce autologous neutralizing responses [1-3]. Recently, a novel triple tandem trimer (TTT) platform has been used to generate a plasmid encoding Env immunogen (pBG505-TTT) that expresses only as trimers, making it more suitable for nucleic acid vaccines. [4]

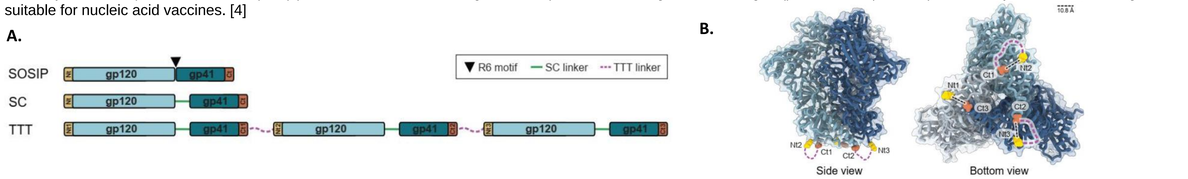


Figure 1: Design of triple tandem trimer Env DNA construct: A. Linear representation of SOSIP, single-chain (SC) and triple tandem trimer (TTT) Env constructs. The SOSIP construct presents a R6 furin cleavage motif (black triangle) between gp120 and gp140 subunits, which is replaced by SC linkers (green lines) in SC and TTT constructs. The TTT construct is composed of three SC protomers fused by TTT linkers (purple lines). N- and C-termini of each protomer are colored in yellow and red, respectively **(A)**. Expected position of TTT linkers on the structure of a BG505.SOSIP trimer **(B)** [4].

2. ADA-1 Enhances TFH Function Making it a Promising Adjuvant for HIV Vaccines

Previous work in our lab has shown that ADA-1 is capable of enhancing T follicular helper (TFH) cell function resulting in increased antibody production from memory B cells [4]. TFH are critical in generating broadly neutralizing antibodies (bnABs) which are needed for an effective HIV immune response (**Figure 2**). For this reason, we believe that ADA-1 would be a promising adjuvant for HIV vaccines.

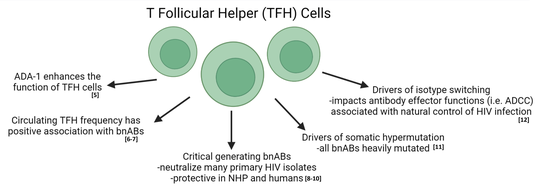


Figure 2: TFH cells are central to generating bnABs

3. ADA enhances HIV specific humoral responses in a dose sparing manner



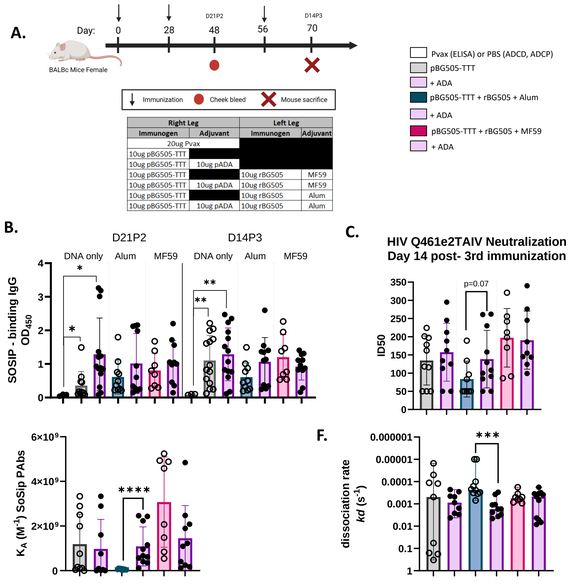
Immunogen	Adjuvant
15ug Pvax	
1ug pBG505-TTT	
1ug pBG505-TTT	10ug pADA
5ug pBG505-TTT	
5ug pBG505-TTT	10ug pADA

ADA coimmunization lead increased magnitude and durability of SOSIP specific antibodies, isotyping switching (IgG1, IgG2a, and IgG2b), and antibody affinity particularly with 1ug of pBG505-TTT. In contrast, the 5ug of pBG505-TTT alone or with pADA had similar responses. The ability of pADA to significantly enhance humoral responses with 1ug of pBG505-TTT to levels comparable with 5ug of pBG505-TTT clearly demonstrates its dose sparing effect.

Figure 3: ADA enhances HIV specific humoral responses in a dose sparing manner. Female BALB/c mice were immunized thrice with 1ug or 5ug of pBG505-TTT alone or with 10ug of DNA-encoded ADA (pADA). Control mice were immunized with 15ug of the empty backbone pVax (- control). Mice were bled at D14P1 and D21P2 immunizations prior to sacrifice at D14P3 immunizations **(A)**. Sera was used to determine SOSIP specific antibody responses via ELISA **(B-I)** and antibody dependent complement deposition (ADCD) **(J)** and antibody dependent cellular phagocytosis (ADCP) **(K)**. Data are representative of one experiment. Symbols represent individual animals, bars represent the mean, error bars represent the standard deviations. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001 by Mann-Whitney-U test **(B-G)** or Kruskal-Wallis ANOVA **(J)**.

4. Higher doses of pBG505-TTT and protein coimmunization are needed to see qualitative differences in HIV specific Abs

Coimmunization with pADA significantly enhanced antibody neutralization compared to mice immunized with pBG505-TTT, rBG505 and alum. We found that antibody quantity did not correlate with neutralization capacity and that ADA coimmunization did not enhance ADCD and ADCP compared to their non-ADA receiving counterparts. Similar analyses with sera from 1ug or 5ug pBG505-TTT immunized mice show little to no neutralization or binding affinity which was not enhanced with pADA.



Right Leg		Left Leg	
Immunogen	Adjuvant	Immunogen	Adjuvant
20ug Pvax			
10ug pBG505-TTT		10ug rBG505	MF59
10ug pBG505-TTT	10ug pADA	10ug rBG505	MF59
10ug pBG505-TTT		10ug rBG505	Alum
10ug pBG505-TTT	10ug pADA	10ug rBG505	Alum

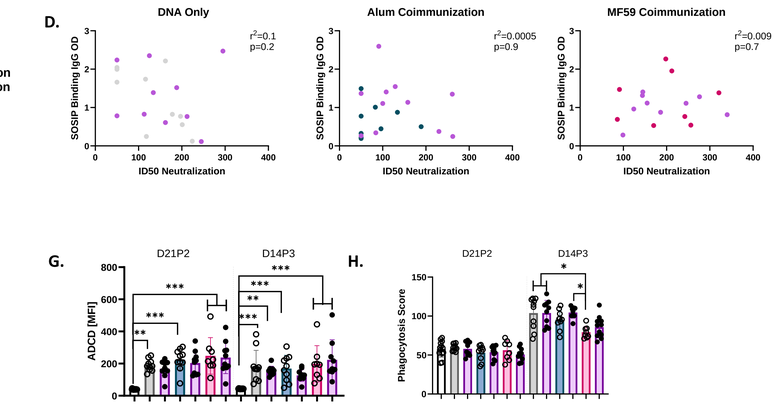


Figure 4: Higher doses of pBG505-TTT and protein coimmunization are needed to see qualitative differences in HIV specific Abs. Mice were immunized with 10ug of pBG505 alone or with 10ug pADA. A separate group of mice were immunized with both DNA as described and 10ug of trimeric Env recombinant protein (rBG505-SOSIP) with adjuvants Alum or MF59. Mice were bled at D21P2 immunizations prior to sacrifice at D14P3 immunizations **(A)**. Sera was used to determine SOSIP specific IgG **(B)**, neutralization **(C)**, correlation of SOSIP IgG to neutralization **(D)**, antibody affinity **(E)** and disassociation rate **(F)**, ADCD **(G)** and ADCP **(H)**. Symbols represent individual animals, bars represent the mean, error bars represent the standard deviations. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001 by Mann-Whitney-U test **(B-C, E-F)** or Kruskal-Wallis ANOVA **(G-H)**.

5. ADA enhances HIV specific cellular responses

ADA coimmunization lead to enhanced HIV specific CD4+ and CD8+ T cell responses. T cells from ADA communized mice had enhanced polyfunctionality, activation, and expression of CD107a.

Figure 5: ADA enhances HIV specific cellular responses. Spleenocytes from immunized mice **(Fig. 3A)** were harvested at D14P3 immunizations and used to evaluate cellular responses via ELISpot assay **(A-B)**, intracellular cytokine staining **(D-E, H-I)**, and an activation induced marker (AIM) assay **(F-G)**. CD107a expression was evaluated via flow cytometry **(C)**. For all assays, spleenocytes were stimulated with peptides spanning the HIV BG505 envelope protein. Symbols represent individual animals, bars represent the mean, error bars represent the standard deviations. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001 by Mann-Whitney-U test **(C-I)** or 2way ANOVA **(B)**.

6. Future Directions

-Sort SOSIP specific memory B cells. Perform BCR sequencing and gene analysis

-Test alternative immunization strategies – DNA prime, protein boost

-Mechanistic studies to understand *in vivo*
- -Which cell types are taking up pADA and expressing
- -Do these cells traffic from the muscle to other secondary lymphoid organs (i.e. lymph nodes and spleen)
- -Does pADA uptake and expression increase expression of costimulatory molecules

-Mechanistic *in vitro* studies to understand the effect of ADA on other antigen presenting cells such as macrophages

Acknowledgements: We would like to acknowledge Inovio for use of the Cellectra device for vaccination. We would also like to acknowledge Dr. Rogier Sanders for providing the pBG505-TTT DNA constructs and rBG505 protein for immunizations.

Funding: This work was funded by the PA Department of Community & Economic Development COVID AID, Relief, and Economic Security (CARES) act, the NIH NIMH award T32 MH079785 (Drexel Component, Principal Investigator Brian Wigdahl; fellowship to G. Cusimano), and the W.W. Charitable Trust Foundation.

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