HLA-II ASSOCIATED VIRAL ADAPTATION IMPACTS CD4+ T-CELL RESPONSES IN HIV-1 VACCINES

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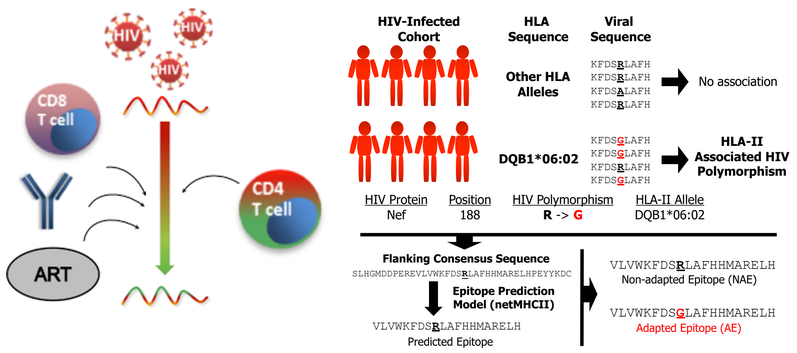
Background



- HLA-II non-adapted epitopes (NAE) have been shown to elicit stronger CD4 immune responses than adapted epitopes (AE) in the context of both acute and chronic HIV-1 infection.[1]
- HIV-1 vaccine inserts commonly encode adapted epitopes (AE) (30-45%, **Figure 1**) that were used in recent clinical trials; however, the impact of these AE on CD4 T cell responses in HIV-1 vaccinees has not been investigated.

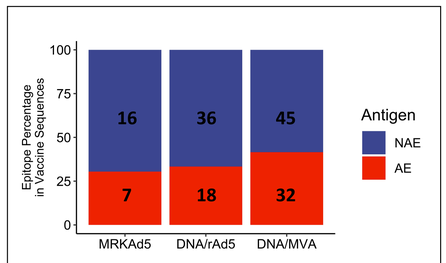
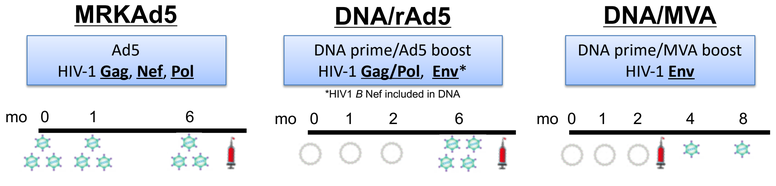


Figure 1: Percentage of adapted and non-adapted epitopes encoded within recent HIV-1 vaccine trials.

HYPOTHESIS: AE-specific CD4 responses will be less immunogenic in comparison to NAE-specific CD4 responses in HIV-1 vaccine recipients.

Approach

- **Samples:** PBMC's (n=88) were obtained from the HVTN across three different HIV-1 vaccine studies.
  - MRKAd5 (HVTN 502): 20 vaccinees, 4 placebos
  - DNA/rAd5 (HVTN 505): 20 vaccinees, 4 placebos
  - Mosaic DNA/MVA (HVTN 106): 30 vaccinees, 10 placebos



- **Antigens:** Sample immunogenicity was tested to a total of 97 non-adapted epitopes (NAE) and 57 adapted epitopes (AE) for each of the three studies above. Epitopes were pooled into 1) (NAE) pools and 2) (AE) pools for each study, unless otherwise stated (Figure 4).
- **Assays:**
  - CD4+ T cell IFNγ ELISpot assay using CD8-depletion
  - Flow-cytometry based activation induced marker (AIM) assay
  - Single-cell RNA sequencing assay

Results

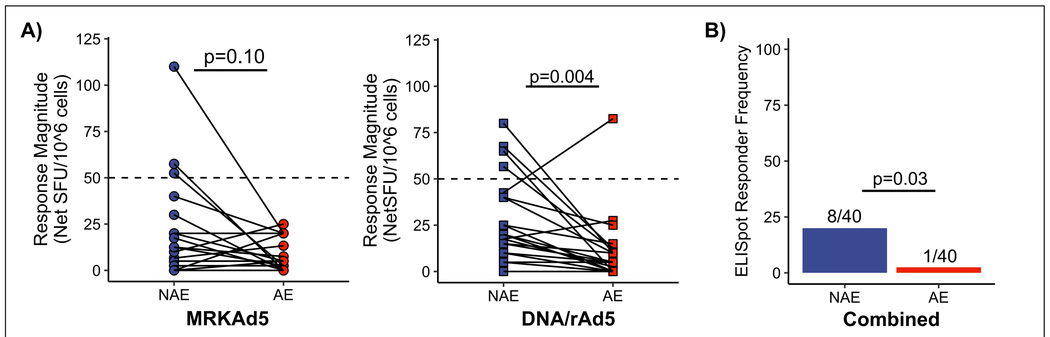


Figure 2: Adapted epitopes are less immunogenic by CD4+ T cell IFNγ ELISpot in HIV-1 vaccine recipients.
A) Net magnitude of CD4+ T cell IFNγ ELISpot responses in HIV-1 vaccinees after stimulation with pooled vaccine-matched NAE or AE in MRKAd5 (n=20) and DNA/rAd5 (n=20). **B)** Overall responder frequency to NAE and AE based on CD8-depleted IFNγ ELISpot responses (positivity criteria: SFU=50+ and at least 3X unstimulated condition).

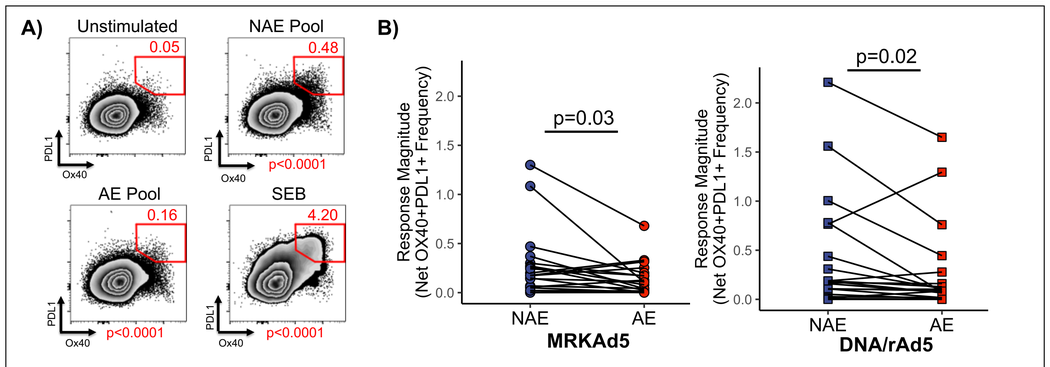


Figure 3: Adapted epitopes induce a lower frequency of activated antigen-specific CD4 T cells based on Ox40/PDL1 co-expression in HIV-1 vaccine recipients.
A) Representative example of AIM Assay and Ox40/PDL1 coexpression in CD4+ T cells from a MRKAd5 vaccinee in response to vaccine-matched peptide pools. **B)** Net magnitude of coexpression of Ox40/PDL1 in response vaccine-matched AE or NAE pools in MRKAd5 (n=20) and DNA/rAd5 (n=20) vaccine recipients.

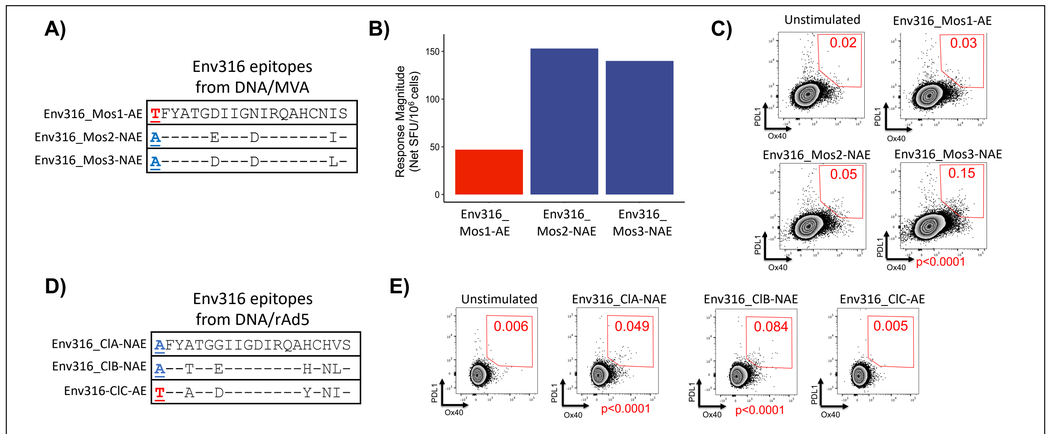


Figure 4: Mosaic DNA/MVA and multi-clade DNA/rAd5 vaccine recipients show decreased immunogenicity to adapted epitopes at the single-epitope level.
A) Predicted epitopes at Env316 site for three mosaic sequences encoded in DNA/MVA vaccine and corresponding **B)** CD4+ T cell IFNγ ELISpot responses and **C)** Ox40/PDL1 coexpression in a single vaccinee. **D)** Similar epitopes for the three clade sequences encoded in DNA/rAd5 vaccine and corresponding **E)** Ox40/PDL1 coexpression in a single vaccinee.

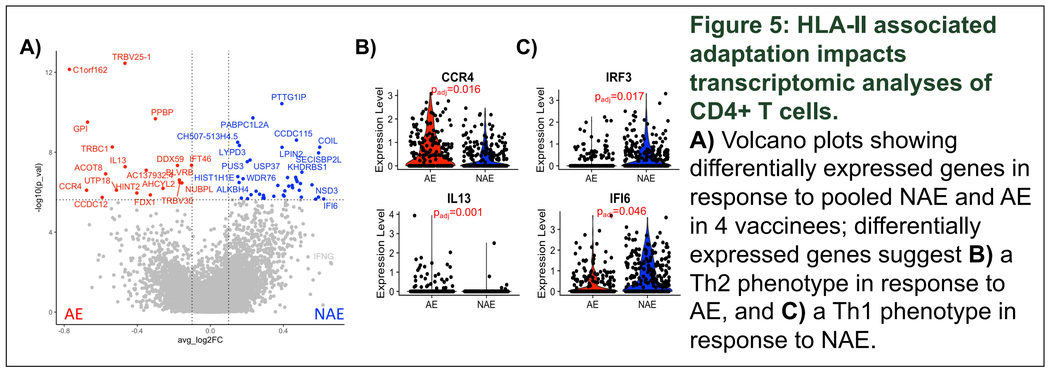


Figure 5: HLA-II associated adaptation impacts transcriptomic analyses of CD4+ T cells.
A) Volcano plots showing differentially expressed genes in response to pooled NAE and AE in 4 vaccinees; differentially expressed genes suggest **B)** a Th2 phenotype in response to AE, and **C)** a Th1 phenotype in response to NAE.

Conclusions

- **Pooled adapted epitopes (NAE) induce a lower frequency and lower magnitude of CD4+ T cell responses in HIV-1 vaccine recipients based on CD4+ T cell IFNγ ELISpot and antigen-specific Ox40/PDL1 coexpression.**
- Vaccine recipients from all HIV-1 vaccine trials tested induced immune responses to predicted epitopes.
- Activation induced marker (AIM) assays elicited more responses when compared to IFNγ ELISpot assays.
- **Single-cell RNA sequencing analysis shows differential gene expression profiles between NAE-specific and AE-specific CD4+ T-cells, with NAE-specific responses favoring a Th1 phenotype and AE-specific responses favoring a Th2 phenotype.**
- These results suggest that future HIV-1 vaccine trials could be optimized by including fully non-adapted CD4+ T-cell epitopes.

Future Directions

- Complete bioinformatics analysis of single-cell RNA sequencing utilizing cellular pathway analysis programs.
- Further define CD4 response functionality by performing intracellular cytokine staining (ICS) and multiplex ELISA assays in response to NAE and AE pools.
- Determine the biological relevance of HLA-II adaptation to HIV-1 by:
  - *in vitro* viral inhibition assay to investigate CD4-mediated viral killing of HIV-1 constructs
  - *in vitro* B cell maturation assay to determine role of CD4s in providing help for B cell maturation.
- Determine whether HLA-II adaptation negatively impacts CD4 responses in other HIV-1 vaccine trials such as ALVAC (HVTN 702) or mosaic vaccines (HVTN 705/706).

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References

1. Erdmann N, Du VY, Carlson J, Schaefer M, Jureka A, Sterrett S, et al. (2015) HLA Class-II Associated HIV Polymorphisms Predict Escape from CD4+ T Cell Responses. PLoS Pathog 11(8): e1005111.
2. Reiss S, Baxter AE, Cirelli KM, Dan JM, Morou A, Daigneault A, et al. (2017) Comparative analysis of activation induced marker (AIM) assays for sensitive identification of antigen-specific CD4 T cells. PLoS ONE 12(10): e0186998.