Cellular Immune Responses in Ugandan Volunteers Enrolled in a Phase 1 HIV-1 DNA (VRC-HIVDNA009-00-VP)/ rAd5 (VRC-HIVADV014-00-VP) Boost Vaccine Trial

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Objectives

To determine the frequency and magnitude of CD4+ and CD8+ T cell responses in freshly isolated peripheral blood mononuclear cells (PBMC) derived from Ugandan HIV seronegative recipients of an adenovirus (rAd5) immunization 22-26 months following priming with HIV-DNA or placebo.

Methods

Vaccine:

The Vaccine Research Center (VRC) DNA-HIV vaccine (VRC-HIVDNA009-00-VP) is composed of 4 circular DNA plasmids, one expressing clade B HIV-1 Gag, Pol and Nef, and the other three expressing clade A, B or C HIV-1 Env. The recombinant adenoviral vector product VRC rAd5-HIV vaccine (VRC-HIVADV014-00-VP) is a replication-deficient, combination vaccine containing a mixture of four recombinant serotype 5 adenoviral vectors. The VRC DNA-HIV vaccine and the VRC rAd5-HIV vaccine contain largely matched HIV gene inserts, but they are not fully identical.

Trial design: (Figure 1)

A multiclade HIV-1 DNA 4-plasmid vaccine (Vaccine Research Center, NIH Bethesda) was tested in a phase I placebo-controlled study in Uganda from 2005-2006 (N=31). The ratio of vaccine to placebo recipients was 1:1 and DNA vaccine was administered at weeks 0, 4 and 8. In 2007, 22-26 months following the final DNA prime, a subset of these volunteers (n=9 vaccinees and n=9 placebos) were boosted with a single recombinant adenovirus-5 (rAd5) vaccine (1010 PU).

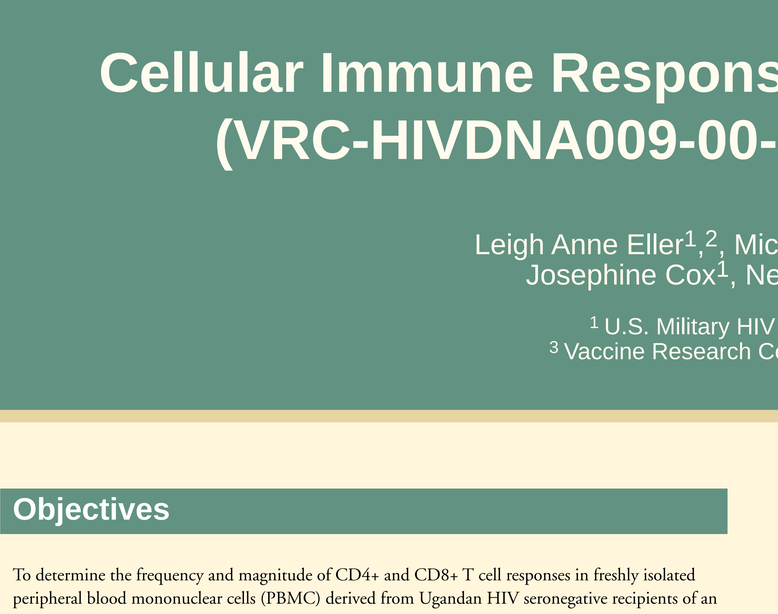
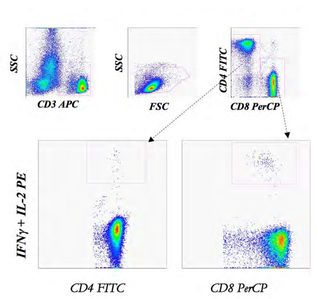


Figure 1

Intracellular Cytokine Staining Assay:

Standard intracellular cytokine staining (ICS) was performed at weeks 0, 6, 24, and 36 weeks following rAd5 boost on freshly isolated PBMC using Interferon-gamma (IFN-γ) / Interleukin-2 (IL-2) four-color flow cytometry. Stimulation of fresh PBMC was done using peptide pools (provided by VRC) for each protein matching the vaccine constructs. Peptides were 15-mers overlapping by 11 aa, representing Env (clade A and B), Pol 1 and Pol 2 (clade B). Peptide pools for Env clade C, Gag clade B and Nef (clade B) were eliminated from assay due to high background reactivity. Peptides were used at a final concentration of 1ug/ml. Each assay included Staphylococcus Entero Toxin B (SEB) and the CD8-restricted CEF pool as positive controls. Negative controls were PBMC supplemented with DMSO matching the DMSO concentration of the peptide pools. Flow cytometric analysis was performed using a FACSCalibur flow cytometer. Approximately 20,000 CD3+/CD8+ and CD3+/CD4+ events respectively were acquired and analyzed using Cell Quest software (Becton Dickinson) or FlowJo Software (Tree-Star Software). Figure 2 shows the gating strategy used. A positive response was defined as ≥ 0.06% and ≥ 0.08% (corrected for unstimulated background) for CD4+ and CD8+ T cells, respectively, and ≥ 3 times background. All assays were conducted blinded to immunization status.

Figure 2: Combined IFN-g + IL-2 Gating Strategy



Ad5 Neutralizing Antibody:

Assays were conducted on baseline serum samples using a validated cell-line based assay by the NVITAL laboratory (Rockville, MD).

Results

Figure 3a: Frequency of Positive responders in DNA prime-rAd5 only group

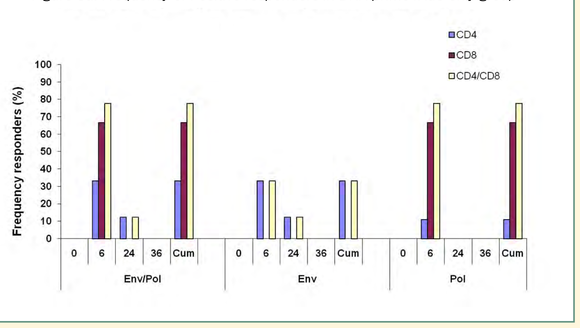


Figure 3a: No positive responses were present on the day of the rAd5 boost. Following immunization, the cumulative response to Env and/or Pol peptides was 7 of 9 participants (78% - 95% CI 40-97%) in the rAd5 arm. Positive responses were elicited in CD4+ (33%: 3/9) and CD8+ (67%: 6/9) T cells to Env and/or Pol at 6 weeks. All CD8+ T cells responses were directed against Pol at week 6 and waned subsequently. CD4+ T cells responses were elicited in 3/9 (33%) and 1/9 (11%) volunteers to Env and Pol, respectively.

Figure 3b: Frequency of Positive responders in DNA prime-rAd5 boost group

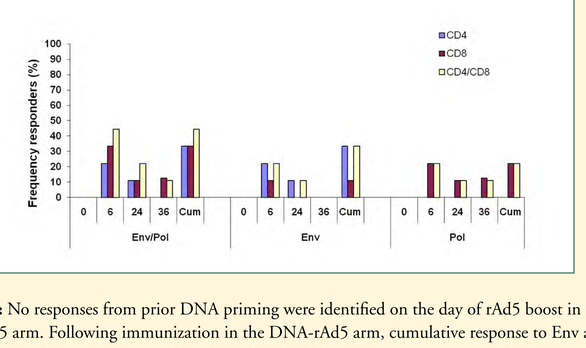


Figure 3b: No responses from prior DNA priming were identified on the day of rAd5 boost in the DNA-rAd5 arm. Following immunization in the DNA-rAd5 arm, cumulative response to Env and/or Pol peptides was 4 of 9 participants (44% - 95% CI 14-79%). Positive responses were elicited in both CD4+ (22%: 2/9) and CD8+ (33%: 3/9) T cells at 6 weeks. In this arm, all observed CD4+ T cells responses were against Env (3/9; 33.3%). CD8+ T cell responses were elicited in 1/9 and 2/9 of volunteers to Env and Pol, respectively.

Figure 4: Median magnitude of all positive responses to Env or Pol peptide pools

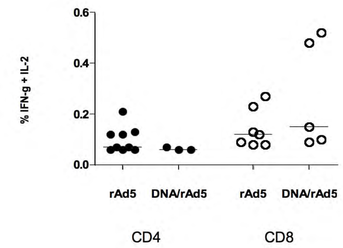


Figure 4: The magnitude of response to either Env or Pol is shown for CD4 and CD8 population split by arm. The magnitude of CD4 or CD8 responses to either Env or Pol was similar in rAd5 alone and DNA/rAd5 groups.

Table 1: Frequency of positive responders by Ad5 titer

	Any Env or Pol		Any Env		Any Pol	
	Ad5 only	DNA/Ad5	Ad5 only	DNA/Ad5	Ad5 only	DNA/Ad5
<12	1/1 (100%) (95% CI:3-100%)	0/1 (0%) (95% CI:0-98%)	0/1 (0%) (95% CI:0-98%)	0/1 (0%) (95% CI:0-98%)	1/1 (100%) (95% CI:3-100%)	0/1 (0%) (95% CI:0-98%)
12-200	0	0	0	0	0	0
201-1000	3/4 (75%) (95% CI:19-99%)	2/3 (66.7%) (95% CI:9-99%)	2/4 (50%) (95% CI:7-93%)	1/3 (33%) (95% CI:1-91%)	3/4 (75%) (95% CI:19-99%)	2/3 (66.7%) (95% CI:9-99%)
>1000	3/4 (75%) (95% CI:19-99%)	2/5 (40%) (95% CI:5-85%)	1/4 (25%) (95% CI:1-81%)	2/5 (40%) (95% CI:5-85%)	3/4 (75%) (95% CI:19-99%)	0/5 (0%) (95% CI:0-52%)

Table 1: Pre-existing Ad5 immunity was observed among 16/18 (89%) volunteers, and titers were >1000 in 10/18 (56%). The frequency of HIV-specific immune responses did not differ significantly based on pre-existing Ad5 titer.

Conclusion

A single administration of rAd5 or DNA priming followed by rAd5 boost after 2 years induced cellular immune responses to HIV antigens, with the former biased to a CD8 response.

Disclaimer

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