



Inguinal Versus Deltoid Vaccination of vCP205 and Blood and Mucosal Immunity to HIV-1

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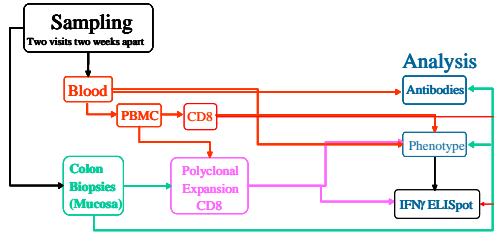
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Background

The ability of vaccines to induce mucosal immunity may be important for protection against HIV transmission. The role of vaccination route in determining systemic and mucosal immune responses is poorly understood. Because inguinal and colonic lymphatics are contiguous, inguinal vaccination may be a strategy to stimulate mucosal immunity. Here we describe the safety and immunogenicity of the vaccine candidate ALVAC-HIV vCP205 used in subcutaneous inguinal and deltoid vaccinations.

Methods

Randomized, double-blinded, and placebo-controlled phase I trial of an intensified vaccination schedule (4 weekly vaccinations). participants received placebo (N=6) or vCP205 (N=12) via deltoid (N=9) or inguinal (N=9) immunization. Samples were obtained at days -28, -14, and 0, followed by vaccinations at days 0, 7, 14 and 21, and collection of experimental samples at days 10, 17, 24, 180, and 365.



- Cell expansions were performed according to published protocols (JT Wong *et al.* 1989. *J. Immunol.* 143: 3404) with the support of J.T. Wong.

- IFN- γ ELISpot: Pools of 12-16 peptides (15mers overlapping by 11 aa) covering the entire coding sequence of HIV-1. 2.3×10^5 cells/well. Positives: 4 times the plate Background and higher than $60 \text{ SFC}/10^6$ Cells.

- Analysis of sero-negative participants revealed that false positives occur under 1.5%.

- As the correlation between visits was ≥ 0.8 , we used the mean of both visits minus the plate background in calculating our results.

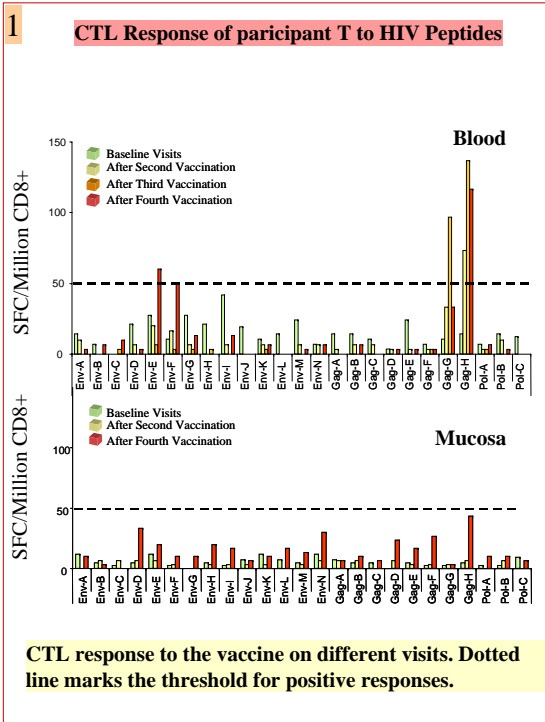
Results

VACCINE: ALVAC™-HIV vCP205 developed by Aventis Pasteur. Canarypox vector expressing Gag, Envelope, and Protease proteins of HIV-1

We present the still-blinded results from 18 volunteers after the fourth vaccination.

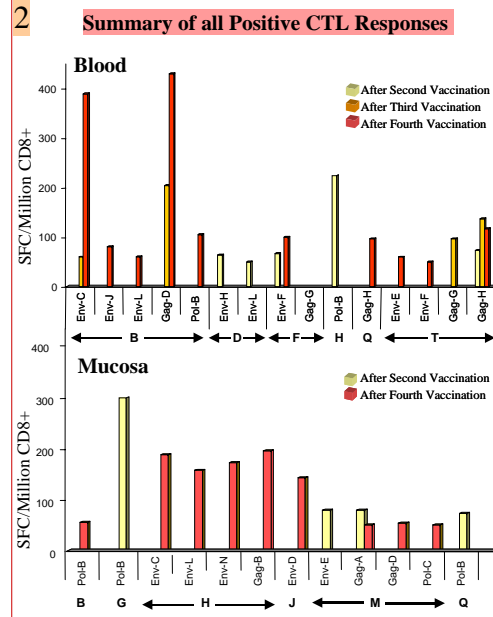
HIV-specific antibodies were undetectable in the serum and mucosal secretions of all participants

No significant increase of activated CD8⁺/CD38⁺ T-cells was observed in blood or mucosa following immunizations.



CTL response to the vaccine on different visits. Dotted line marks the threshold for positive responses.

Results Continued



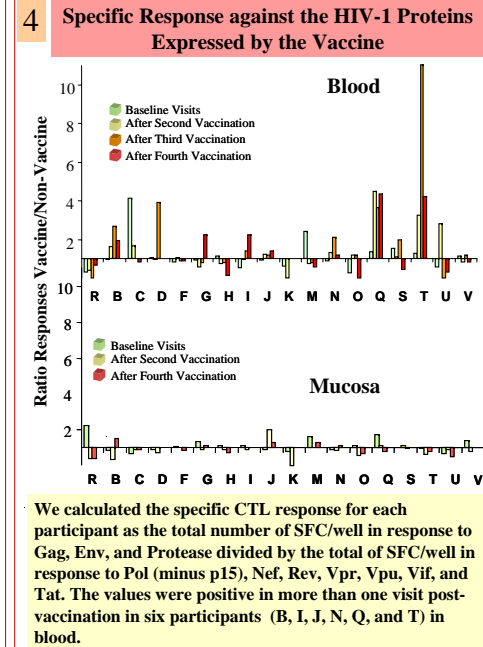
Only participants B, H, and Q presented positive responses in both compartments. participants D, F, and T responded only in blood. participants G, J, and M responded only in mucosa.

3 **Table of Positive Pools**

Subject	PBMC		Expanded PBMC		MMC	
	Fresh Single	Repeat	Fresh Single	Repeat	Expanded Single	Repeat
APB						
APC			3	2	1	
APD						
APF			3			
APG	1		1	1	3	
APH			1		1	
API					4	
API					1	
APK						
APM					3	1
APN						
APO						
APQ			1		1	
APR						
APS						
APT			3	1		
APU						
APV						

Number of positive HIV-1 peptide pools detected for each set of cells (PBMC Fresh and Expanded, and Mucosa expanded-MMC) for all the visits post-vaccination. Single columns: number of positive pools in any single visit. Repeat columns (Red): number of positive pools that could be detected in more than one visit for a given participant. Only one participant, B, responded to the same pool (Pol B) in blood and mucosa in the same visit.

Results Continued



We calculated the specific CTL response for each participant as the total number of SFC/well in response to Gag, Env, and Protease divided by the total of SFC/well in response to Pol (minus p15), Nef, Rev, Vpr, Vpu, Vif, and Tat. The values were higher in more than one visit post-vaccination in six participants (B, I, J, N, Q, and T) in blood.

Summary

After non-specific, polyclonal expansion of CD8⁺ PBMC, HIV-1 positive CTL responses were found in 9 participants: 3 had CTL responses only in PBMC, 3 had responses only in MMC, and 3 had responses in both compartments. In 4 cases we had reproducible responses over separate visits. 10/18 showed a global increase in PBMC CTL against HIV-1 proteins in the vaccine, while 2/18 showed an increase in expanded CD8⁺ MMC HIV-1 specific CTL. Vaccination was also associated with a minor increase in HLA DR⁺/CD38⁺ PBMC CD8⁺ T cells in 6/18, but not in MMC.

Conclusions

vCP205 given on an intensified schedule induces HIV-1-specific CTL responses detectable in the expanded CD8⁺ T-cells from blood. Mucosal responses were fewer in number and lower in magnitude than in blood. Examining CTL responses to all vaccine pools compared to responses to non-vaccine pools appears to be a stringent method of defining positive responses and points to the problems and pitfalls inherent in the use of threshold values to establish positive responses during the analysis of ELISpot assays. Important issues to be addressed when the data are unblinded include whether vaccination near inguinal lymph nodes affects the responsiveness of cellular immunity in the peripheral blood and colonic mucosa.