

AIDSVAX Immunization Induces HIV-specific CD8+ T-cell Responses in High-risk, HIV-negative Volunteers Who Subsequently Acquire HIV Infection

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Abstract

VAX004, the first phase III efficacy trial of an HIV vaccine, was a randomized, double-blinded, placebo-controlled trial which completed in 2003 without clear demonstration of effectiveness in the reduction of either the acquisition of infection or level of plasma viremia after HIV infection. The vaccine consisted of 300ug each of rgp120 envelope subunits derived from two subtype B isolates (AIDSVAX B/B; VaxGen). We evaluated whether T-cell responses can be elicited from VAX004 vaccine recipients.

Cryopreserved PBMC were randomly selected from placebo and vaccine recipients based on behavioral risk factors documented at enrollment. Behavioral risk scores ranged from 0 to 7; and were categorized as either low (0-2) or high (3-7). T-cell proliferation was measured by flow cytometry using carboxyfluorescein diacetate, succinimidyl ester (CFSE)-labeled PBMC.

Valid data were obtained for 149 samples. CD8+ HIV-specific T-cell proliferation was detected in 15/87 (17.2%) of individuals who received the vaccine compared to 1/62 (1.6%) placebo recipients. HIV-specific responses were more frequently detected among vaccinees in the high-risk (N=34) compared to low-risk (N=53) groups (26.5% and 11.3%, respectively) but did not reach statistical significance (OR 2.82, p-value=0.075). Surprisingly, the frequency of responses was higher in vaccine recipients who subsequently acquired HIV infection compared to individuals who remained HIV seronegative (34.8% and 10.9%, respectively, OR 4.34, p-value=0.013). This difference remains statistically significant after adjusting for risk score (OR 4.05, p-value=0.020). No difference in the frequency of HCMV-specific proliferation was observed among all subgroups (p>0.05).

Our preliminary data indicate that HIV-specific T-cells can be induced in HIV uninfected volunteers receiving recombinant gp120 HIV vaccine in the VAX004 study. The immune response rate appears to be highest in high-risk participants who would go on to contract HIV infection. These results suggest that AIDSVAX may boost preexisting cellular immune responses—due to pre-infection exposure. However, vaccine-induced enhancement of HIV infection cannot be ruled out.

Methods

Study Samples

Cryopreserved PBMC were randomly selected from placebo and vaccine recipients based on behavioral risk factors documented at enrollment. Behavior risk score ranged from 0 to 7; and was categorized as either low (0-2) or high (3-7). Only samples with PBMC viability of ≥75% were subsequently tested and all assays were performed blinded to the randomization status. All study volunteers were HIV seronegative at the time of blood collection.

Antigens

Peptides corresponding to the vaccine sequences of the clade B HIV-1 Env gp120 MN were synthesized as 15amino acids (a.a.) overlapping by 1 a.a. (NIH/NIAID repository). A single pool of overlapping peptides, corresponding to the amino acid sequence of the PP65 protein (JPT Peptide Technologies) was used to detect human CMV (HCMV)-specific responses the final concentration of individual peptides was 1ug/ml per peptide.

CFSE Proliferation Assay

Cell proliferation was determined by carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution using the CellTrace™ CFSE Cell Proliferation Kit (Invitrogen, Carlsbad, CA) as per manufacturer's instructions. Cells were cultured for five days at 37°C and 5% CO₂, then harvested and stained for surface markers with the following antibodies: CD3 APC, CD4 PE, CD8 PerCp-Cy5.5. A minimum of 30,000 CD3+ cells per sample were acquired using a 4-color flow cytometer (FACS Calibur, BD Biosciences). All flow analysis was performed by FlowJo software (TreeStar, San Carlos, CA). Proliferation was measured by the extent of CFSE dilution. Results greater than twice the background values and more than 0.1% after subtraction of background were considered positive. Staphylococcal Enterotoxin B (SEB Sigma-Aldrich, St. Louis, MO) stimulation was used as a positive control. All study participants demonstrated significant proliferation following SEB stimulation.

Statistical Analysis

Logistic regression analysis was used to model the log odds ratio for a positive CD8+ response adjusting for 1) risk category; 2) Subsequent HIV infection status and 3) Subsequent HIV infection status and risk score. Statistical significance was defined as p <0.05.

Env-specific Responses

Participants	Vaccine (N=87)	Placebo (N=62)
High risk	9/34	1/30
Low Risk	6/53	0/32
Odd ratio	2.82	
(95% CI)	[0.91, 9.28]	
p-value	0.075	NS

Vaccine Responders

Outcome	Vaccine Recipient N=87	Percent Response **
Uninfected	7/64	10.9%
HIV infection	8/23	34.8%
Odd ratio **		4.34
(95% CI)		[1.36, 14.34]
p-value		0.013

** OR adjusted for risk score is 4.05 [1.24, 13.55], p=0.020

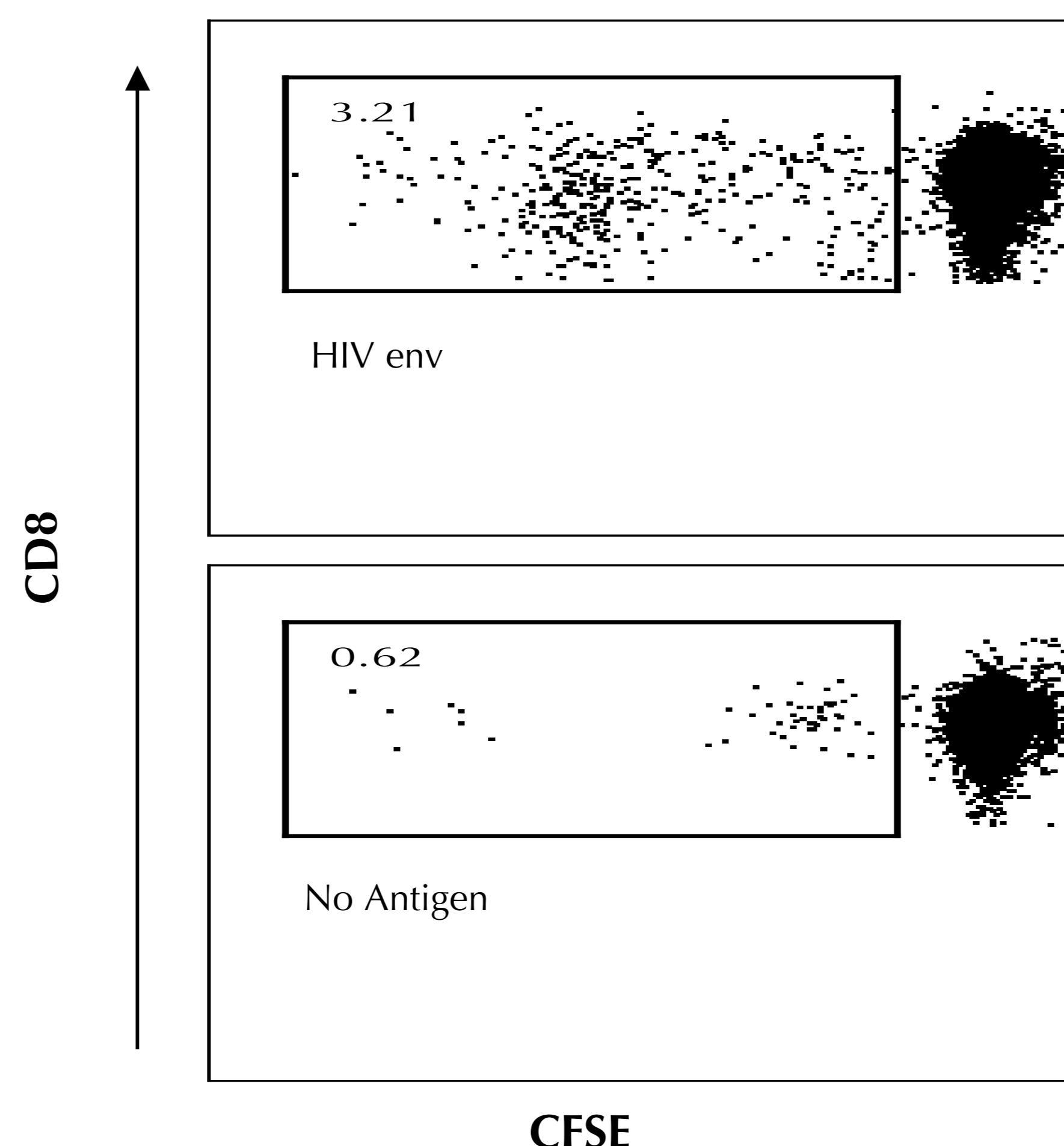


Figure 1: Representative Plot of ENV-specific CD8 proliferation. CFSE-labeled PBMC were stimulated with ENV peptides for 5 days then assessed for proliferation by flow cytometry. Results are expressed as percent of proliferating CD8+ T-cells as measured by the extent of CFSE dilution. Positive proliferation is defined as >0.1% net and at least twice background.

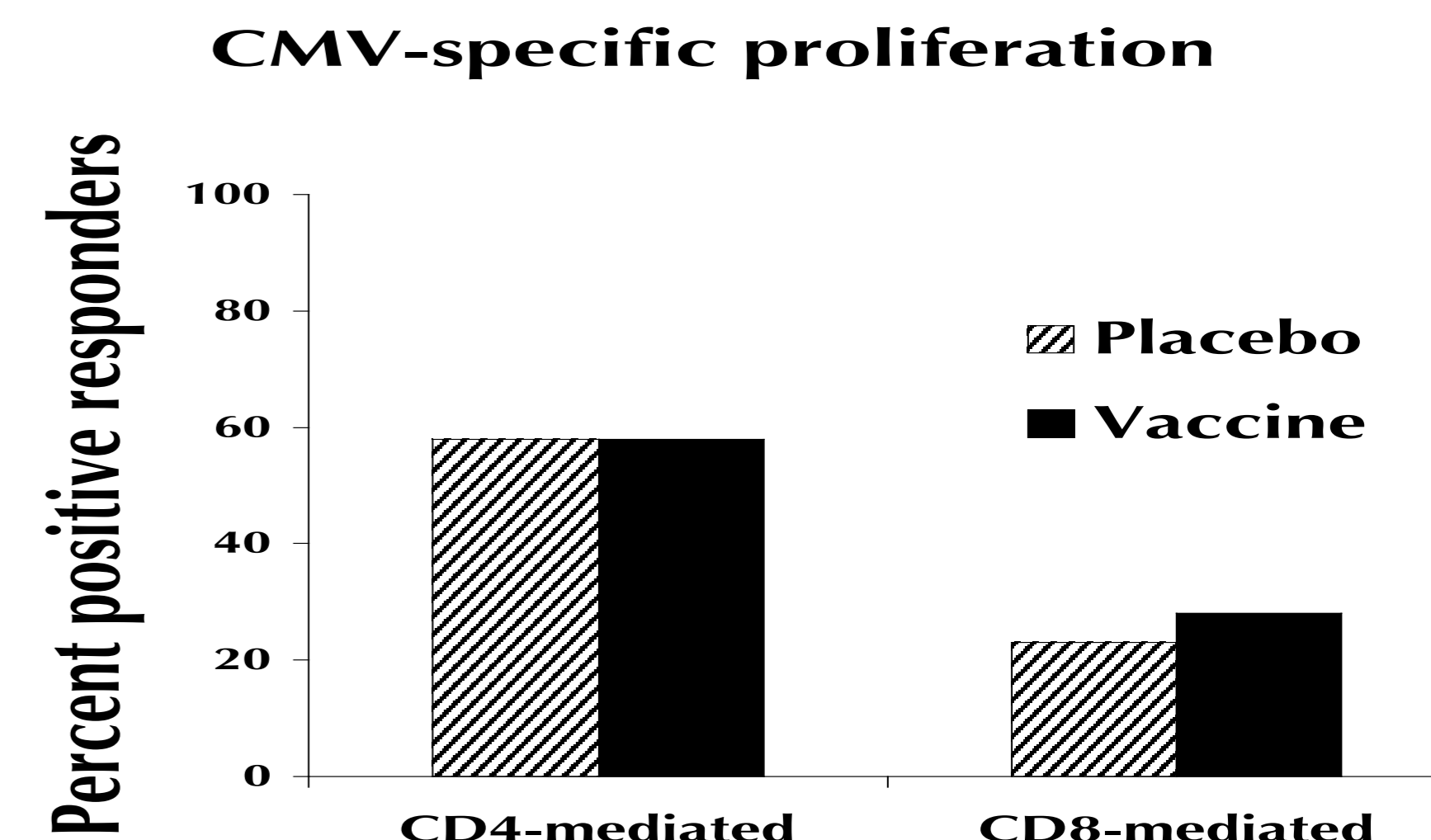


Figure 2: CMV-specific CD4+ and CD8+ proliferation in VAX004 samples. CFSE-labeled PBMC were stimulated with HCMV peptides then assessed for proliferation after 5 days. No significant difference between the frequency of responses in CD4- and CD8- mediated proliferation was seen between placebo and vaccine recipients (p.0.05)

Summary and Conclusions

1. Vaccine-specific CD8+ T-cells can be induced in HIV-uninfected volunteers vaccinated with rgp120 AIDSVAX
2. No significant differences in Env-specific CD8+ T-cell responses were observed between high risk and low risk vaccine recipients
3. Env-specific CD8+ T-cell responses was significantly higher in vaccine recipients who eventually acquired HIV infection, regardless of behavioral risk factors
4. Our data suggest that AIDSVAX may boost preexisting cellular immune responses—due to pre-infection exposure. However, vaccine-induced enhancement of HIV infection cannot be ruled out.