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COMPARATIVE ANALYSIS OF THE MAGNITUDE AND QUALITY OF VACCINE-ELICITED T-CELL RESPONSES

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Background & Aim

To date, six vaccine strategies have been evaluated in clinical trials for their efficacy at inducing protective immune responses against HIV infection. However, only the ALVAC-HIV/AIDSVAX B/E vaccine (RV144 trial) has demonstrated protection, albeit modestly (31%; P 0.03). One potential correlate of protection was a low-frequency HIV-specific CD4 T cell population with diverse functionality. Although CD4 T cells, particularly T follicular helper (Tfh) cells, are critical for effective antibody responses, most studies involving HIV vaccines have focused on humoral immunity or CD8 T cell effector responses, and little is known about the functionality and frequency of vaccine-induced CD4 T cells. We therefore assessed antigen-specific CD4 memory responses from several phase I/II clinical trials and compared them to responses to natural HIV-1 infection. These trials utilized different vaccination strategies, modes of vaccine delivery, and prime/boost combinations, and our aim was to find out if these modalities have an influence on the functional profile of the CD4 T cells induced.

Methods

We characterized the functional profiles of CD4 T cells using multicolor flow cytometry on cryopreserved PBMCs isolated from chronically HIV-infected patients or HIV vaccination study participants. The phase I/II clinical vaccination trials included in this analysis were RV138, RV172, RV114, RV132, RV135, RV158, and RV144. Cryopreserved PBMCs were thawed, restimulated with either overlapping HIV-1 Env or Gag peptide pools, live/dead-stained, surface stained for CD3, CD4, CD8, CXCR5, PD-1, CD40L, CD107A and CD45RO and intracellular staining was performed for IL-21, IFNγ, TNFα.

Conclusion

- Human CD4 T cell functional profiles are significantly different when comparing immune responses in chronic HIV infection to those induced by several HIV vaccination trials
- The choice of vaccine components in combination with the choice of the vaccine delivery route altered both quality and quantity of the CD4 memory T cell responses
- We characterized the first HIV vaccination trial showing any protection (RV144) as eliciting a polyfunctional response

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Results

I) The CD4 T cell memory response differs strongly between HIV-1 vaccination and chronic HIV-1 infection

- CD4 T cell responses differ markedly between chronic HIV-infected patients and subjects who received HIV vaccines. Chronically infected patients display a polyfunctional response dominated by TNFα and CD40L-positive memory CD4 T cells
- Vacinees have much lower frequencies of CD107A, IFNγ, IL21, and TNFα, but similar amounts of CD40L+ cells
- The frequency of lymph node homing receptor CXCR5-expressing cells is upregulated in all vaccinee groups compared to chronically infected patients

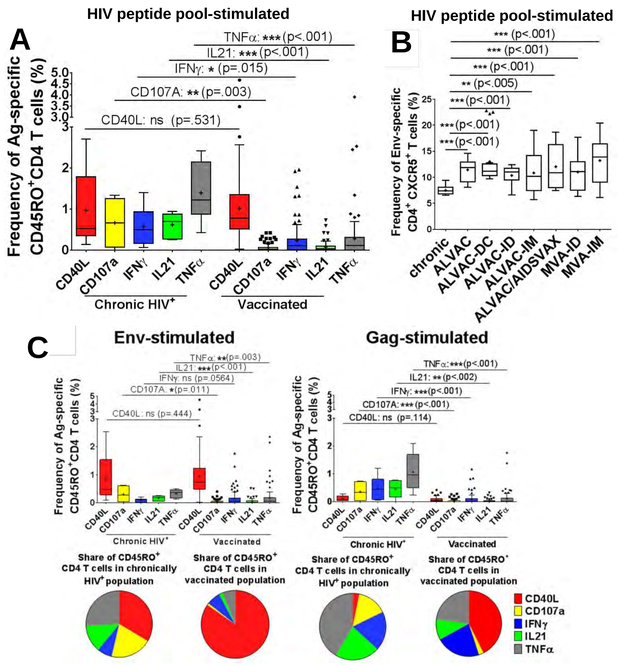

Figure 1: (A) **Fig. 1: Total frequency of the immunological response to HIV potential T cell epitope peptide stimulation in memory T cells. (A)** Blood was drawn from chronically HIV-infected patients. In parallel, blood was collected 24 weeks after vaccination with ALVAC-HIV encoding Gag and Env. PBMCs isolated from the blood were stimulated with HIV peptide pools and analyzed via flow cytometry for the frequency of Env/Gag specific memory T cells (CD45RO$^+$) expressing either CD40L, CD107a, IFNγ, IL-21, or TNFα. **(B)** PBMCs from chronically HIV-infected (chronic, n=6) or ALVAC-HIV vaccinated patients (vaccines, n=97) were stimulated with either HIV Env or Gag potential T cell peptide pools and the response measured as described above. Each experiment was repeated with blood from at least 6 donors. **(C)** PBMCs from uninfected (n=6), chronically HIV-positive (n=18), and vaccine trial patients (ALVAC n=21, ALVAC-DC n=18, ALVAC-ID n=21, ALVAC-IM n=21 ALVAC/AIDSVAX n=18, MVA-ID n=39, MVA-IM n=30) were compared by the frequency of CXCR5-expressing CD4$^+$ T cells after Env stimulation (results for Gag were similar, data not shown). For statistical analysis the Mann-Whitney U test was performed and the asterisk representation of p values is shown in NEJM style.

II) Choice of immunogen and vaccine delivery route decides the functional profile of CD4 memory T cells

- The combination of different DNA vector primes with varying routes of vaccine administration produced subtle but significant differences in the resulting CD4 memory T cell function profile
- When only comparing intradermal (ID) vs. intramuscular (IM) injection no differences in functional responses was observed

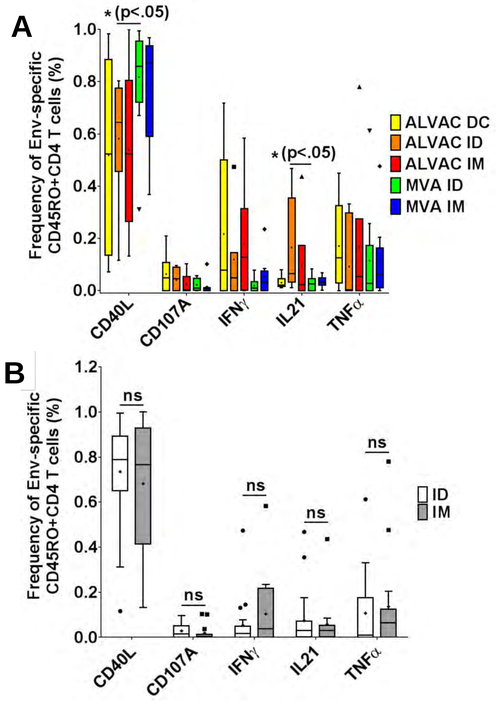

Figure 2: **Effect of vaccine administration route on overall frequency to stimulation with HIV Env peptides and on the contribution of each cytokine to the total response. (A)** 24 weeks after ALVAC (Canarypox vector-based) or MVA (Modified Vaccinia Ankara vector-based) vaccination was given either via different routes (intradermally, for MVA n=13, ALVAC n=7 or intramuscularly, MVA n=9, ALVAC n=6) or in the case of ALVAC with autologous DCs (n=6), PBMCs from peripheral blood were analyzed for the frequency of Env-specific memory T cells by flow cytometry. After statistical analysis of all data via the Mann-Whitney U test only significant differences were labelled with the significance level and the corresponding p value. **(B)** The Env-specific responses to Modified Vaccinia Ankara vector-based vaccine delivered intradermally (n=20) or intramuscularly (n=15) were analyzed as to how much the molecules CD40L, CD107a, IFNγ, IL-21, or TNFα contribute to them. Each experiment was repeated with blood from at least 6 donors and the data analyzed for statistically significant differences by the Mann-Whitney U test.

III) A polyfunctional CD4 T cell response can be induced by the right choice of DNA prime, gp160 protein boost, and adjuvant

- The two vaccination stragies eliciting the most varied polyfunctional response were ALVAC vector prime + oligomeric gp160 boost and ALVAC vector prime +bivalent gp120 boost (AIDSVAX)
- While responses to some vaccination strategies werde dominated by CD40L+ cells more polyfunctional responses also featured a rise in IFNγ and TNFα-expressing cells

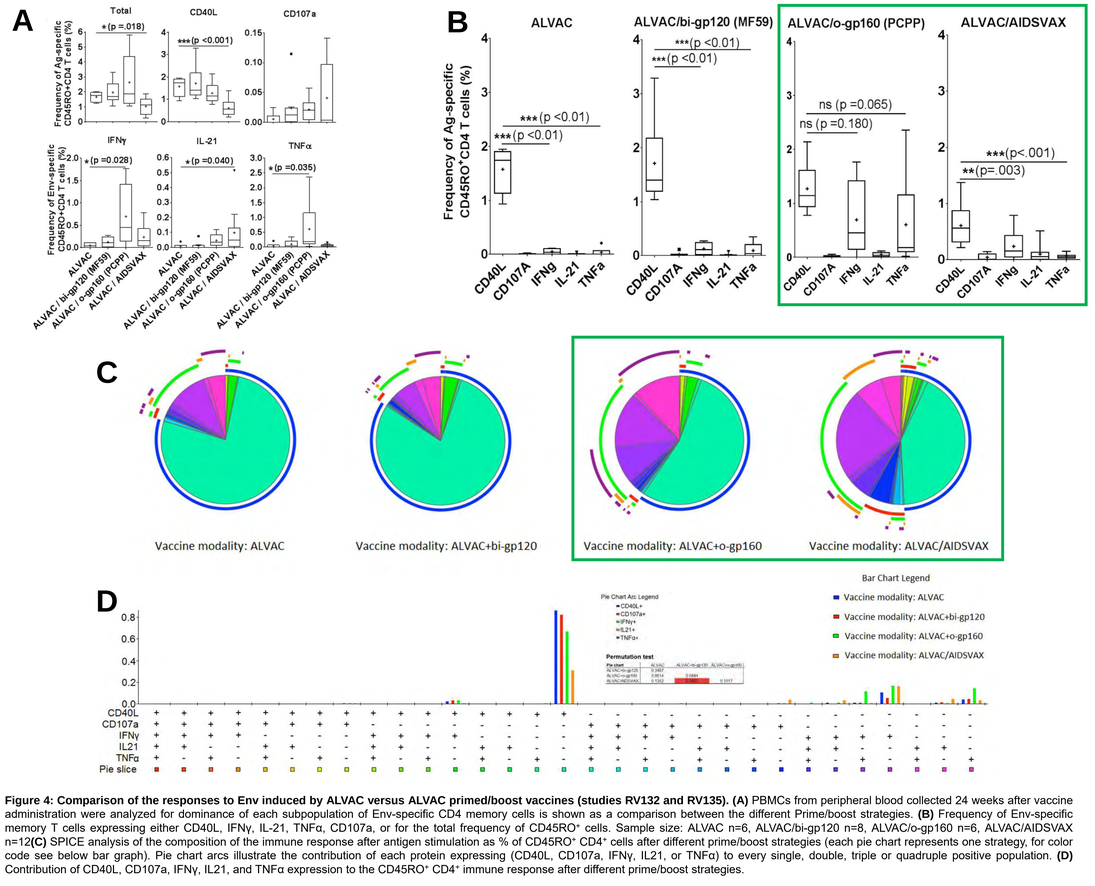

Figure 4: Comparison of the responses to Env induced by ALVAC versus ALVAC primed/boost vaccines (studies RV132 and RV135). (A) PBMCs from peripheral blood collected 24 weeks after vaccine administration were analyzed for dominance of each subpopulation of Env-specific CD4 memory cells is shown as a comparison between the different Prime/boost strategies. **(B)** Frequency of Env-specific memory T cells expressing either CD40L, IFNγ, IL-21, TNFα, CD107a, or for the total frequency of CD45RO$^+$ cells. Sample size: ALVAC n=6, ALVAC/bi-gp120 n=8, ALVAC/o-gp160 n=6, ALVAC/AIDSVAX n=12**(C)** SPICE analysis of the composition of the immune response after antigen stimulation as % of CD45RO$^+$ CD4$^+$ cells after different prime/boost strategies (each pie chart represents one strategy, for color code see below bar graph). Pie chart arcs illustrate the contribution of each protein expressing (CD40L, CD107a, IFNγ, IL21, or TNFα) to every single, double, triple or quadruple positive population. **(D)** Contribution of CD40L, CD107a, IFNγ, IL21, and TNFα expression to the CD45RO$^+$ CD4$^+$ immune response after different prime/boost strategies.