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# Comparative analysis of SIV-specific cellular immune responses induced by different vaccine platforms in rhesus macaques

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**Abstract** To identify the most promising vaccine candidates for combinatorial strategies, we compared five SIV vaccine platforms including recombinant canary pox virus ALVAC, replication-competent adenovirus type 5 host range mutant RepAd, DNA, modified vaccinia

*Abbreviations:* PBMCs, peripheral blood mononuclear cells; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ; IM, intramuscular; IN, intranasal; O, oral; IT, intratracheal; IR, intrarectal; ADCC, antibody-dependent cell-mediated cytotoxicity; ADCVI, antibody-dependent cell-mediated viral inhibition.

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DNA;
Protein;
Pox virus ALVAC MVA;

Ankara (MVA), peptides and protein in distinct combinations. Three regimens used viral vectors (prime or boost) and two regimens used plasmid DNA. Analysis at necropsy showed that the DNA-based vaccine regimens elicited significantly higher cellular responses against Gag and Env than any of the other vaccine platforms. The T cell responses induced by most vaccine regimens disseminated systemically into secondary lymphoid tissues (lymph nodes, spleen) and effector anatomical sites (including liver, vaginal tissue), indicative of their role in viral containment at the portal of entry. The cellular and reported humoral immune response data suggest that combination of DNA and viral vectors elicits a balanced immunity with strong and durable responses able to disseminate into relevant mucosal sites.


## 1. Introduction

To date, four clinical efficacy trials against HIV have been conducted including: (i) gp120 protein vaccine (VaxGen) [1–4]; (ii) recombinant Ad5 (STEP) [5–7]; (iii) DNA prime-recombinant Ad5 boost (HVTN 505) [8]; (iv) combination of recombinant Canarypox ALVAC®-HIV (vCP1521; containing Gag, PR and Env) with gp120 Env protein (AIDSVAX® B/E) (referred to as RV144, conducted in Thailand) [9]. Only the RV144 trial showed modest statistically significant protection from infection [9]. This trial revealed a critical role of humoral responses in preventing infection [10–14]. The humoral immune response waned rapidly after vaccination, indicating the need for vaccine regimens that provided longer-lasting immunity. In addition, no difference in the levels of viremia were found between infected vaccinees and unvaccinated controls, indicating suboptimal cellular immune responses induced by this vaccine protocol. Thus, there is a need to develop a vaccine regimen against HIV that is able to provide effective humoral responses to prevent virus acquisition as well as potent cytotoxic effector memory T cell responses able to contain infection. Importantly, it is critical that humoral and cellular responses disseminate efficiently to mucosal sites (rectum, vagina), since these are portals of entry for HIV infection.

The five sections of the National Cancer Institute's Vaccine Branch have been studying distinct vaccine regimens, which have shown some degree of protection from virus acquisition and/or significant control of peak and/or chronic viremia such as: (i) ALVAC/Env vaccine using a recombinant canary pox virus (ALVAC) vector in combination with an Env protein boost delivered via the intramuscular route (IM) [15–19] (Vaccari M. et. al., manuscript in preparation); (ii) RepAd/Env vaccine consisting of mucosal priming by replication-competent adenovirus type 5 host range mutant recombinants (RepAd) followed by an IM-delivered Env protein boost [20–25]; (iii) DNA vaccine delivered via the IM route followed by electroporation (EP) [26–33]; (iv) DNA&Env vaccine consisting of DNA and Env protein co-immunization delivered as in (iii) [31,32]; and (v) IL-15-adjuvanted viral-specific peptides given together with a TLR agonist delivered intrarectally in combination with recombinant modified vaccinia Ankara (MVA) vectors and Env protein [34–37]. In a comparative study, we tested these five vaccine regimens side-by-side in rhesus macaques and we have recently reported on our comparison and characterization of the humoral responses induced by these platforms [38]. We found that the ALVAC/Env, RepAd/Env and DNA&Env regimens induced robust systemic binding antibodies with neutralizing activity and able to mediate antibody-dependent cellular cytotoxicity (ADCC) and opsonization. Mucosal IgA and IgG responses were readily detected in animals vaccinated with ALVAC/Env, RepAd/Env, DNA&Env and DNA at necropsy, but the RepAd/Env regimen induced the earliest mucosal SIV-specific IgA responses.

Several lines of evidence support the importance of cellular responses for the control of viral propagation in HIV-infected individuals. Some studies reported an association between CTL responses against HIV proteins and control of viremia [39–46]; other studies demonstrated that high avidity CTLs targeting strictly conserved viral regions are preferentially found in HIV-infected controllers and long-term non-progressors [47,48]. Similarly, a correlation between vaccine-induced cellular responses and improved control of viremia has also been described using the SIV/rhesus macaque model [22,27,32,49–64]. Among the vaccine platforms studied in our branch, a correlation between vaccine-induced cell-mediated responses and reduction of viremia was found in DNA immunized animals challenged with SIVmac251 [27,62], in DNA and DNA&Env immunized macaques challenged with SIVsmE660 [32], in DNA-ALVAC immunized animals challenged with SIVmac251 [19], in RepAd/Env vaccinated animals challenged with $SIV_{mac251}$ [22,63,64] and upon intrarectal peptide and MVA vaccine vaccination challenged with $SIV_{mac251}$ or SHIVKu2 [34–37]. The referred vaccination regimens also induced humoral responses against Env, therefore it was unclear whether vaccine-induced T cell responses only, in the absence of humoral responses, were sufficient to mediate control of viremia. However, several studies in macaques unequivocally demonstrated the efficacy of T cell responses in controlling highly pathogenic SIVmac: (i) animals vaccinated with recombinant CMV expressing SIV antigens controlled viremia to undetectable level in the presence of vaccine-induced CTL responses and absolute absence of anti-SIV humoral responses [65–67]; (ii) macaques vaccinated with immunogens lacking an Env component were able to significantly control viremia [68–72]. In the present study, we report a comparison of systemic cellular immune responses induced by the different vaccine platforms being explored in our Vaccine Branch, which may provide suggestions for combinations that further optimize vaccine regimens.