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MAJOR ARTICLE

Randomized, Double-Blind Evaluation of Late Boost Strategies for HIV-Uninfected Vaccine Recipients in the RV144 HIV Vaccine Efficacy Trial

Supachai Rerks-Ngarm,[1,a] Punnee Pitisuttithum,[2,a] Jean-Louis Excler,[4,5,c] Sorachai Nitayaphan,[3] Jaranit Kaewkungwal,[2] Nakorn Premsri,[1] Prayura Kunasol,[1] Nicos Karasavvas,[3] Alexandra Schuetz,[3,4,5] Viseth Ngauy,[3,d] Faruk Sinangil,[7] Peter Dawson,[6] Allan C. deCamp,[8] Sanjay Phogat,[9] Sanjay Garunathan,[9] James Tartaglia,[9] Carlos DiazGranados,[9] Silvia Ratto-Kim,[4,e] Poonam Pegu,[4,5] Michael Eller,[4,5] Chitraporn Karnasuta,[3] David C. Montefiori,[10,11] Sheetal Sawant,[10] Nathan Vandergrift,[10] Saintedym Wills,[10] Georgia D. Tomaras,[10] Merlin L. Robb,[4,5] Nelson L. Michael,[4] Jerome H. Kim,[4,c] Sandhya Vasan,[3,4,5,b] and Robert J. O'Connell[3,4,b]; for the RV305 Study Team[f]

[1]Department of Disease Control, Ministry of Public Health, Nonthaburi, [2]Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University, and [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; [4]US Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, [5]Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, and [6]The Emmes Corporation, Rockville, Maryland; [7]Global Solutions for Infectious Diseases, South San Francisco, California; [8]Vaccine and Infectious Disease Division and Statistical Center for HIV/AIDS Research and Prevention, Fred Hutchinson Cancer Research Center, Seattle, Washington; [9]Sanofi Pasteur, Swiftwater, Pennsylvania; [10]Duke Human Vaccine Institute and [11]Department of Surgery, Duke University, Durham, North Carolina

Background. The RV144 ALVAC-HIV prime, AIDSVAX B/E boost afforded 60% efficacy against human immunodeficiency virus (HIV) acquisition at 1 year, waning to 31.2% after 3.5 years. We hypothesized that additional vaccinations might augment immune correlates of protection.

Methods. In a randomized placebo-controlled double-blind study of 162 HIV-negative RV144 vaccine recipients, we evaluated 2 additional boosts, given 6–8 years since RV144 vaccination, for safety and immunogenicity, at weeks 0 and 24. Study groups 1–3 received ALVAC-HIV+AIDSVAX B/E, AIDSVAX B/E, and ALVAC-HIV, respectively, or placebo.

Results. Vaccines were well tolerated. For groups 1 and 2, plasma immunoglobulin (Ig) G, IgA, and neutralizing antibody responses at week 2 were all significantly higher than 2 weeks after the last RV144 vaccination. IgG titers against glycoprotein (gp) 70V1V2 92TH023 increased 14-fold compared with 2 weeks after the last RV144 vaccination (14 069 vs 999; $P < .001$). Groups 1 and 2 did not differ significantly from each other, whereas group 3 was similar to placebo recipients. Responses in groups 1 and 2 declined by week 24 but were boosted by the second vaccination, albeit at lower magnitude than for week 2.

Conclusions. In RV144 vaccinees, AIDSVAX B/E with or without ALVAC-HIV 6–8 years after initial vaccination generated higher humoral responses than after RV144, but these responses were short-lived, and their magnitude did not increase with subsequent boost.

Clinical Trials Registration. NCT01435135.

Keywords. HIV; vaccine; RV144; prime-boost.

Although 5 human immunodeficiency virus (HIV) preventive vaccine trials failed to demonstrate efficacy [1–5], ALVAC-HIV (vCP1521) boosted by AIDSVAX B/E in RV144 demonstrated 31.2% efficacy after 3.5 years [6] and 60% 1 year after vaccination in post hoc analysis [7]. Binding of plasma immunoglobulin (Ig) G antibodies to variable regions 1 and 2 (V1V2) HIV-1 envelope (Env) proteins correlated inversely with infection risk, and binding of plasma IgA antibodies to Env proteins correlated directly with infection without enhancement [8]. Further post hoc analyses elucidated the importance of V2 and V3 responses [9–13], polyfunctional $CD4^+$ T cells [14], IgG subclasses mediating cellular functions [15, 16], HLA class II association [17], and other non-neutralizing mechanisms [18, 19]. Because antibody responses waned rapidly after vaccination [13], we hypothesized that additional boosts given to RV144 vaccinees might augment responses inversely correlated with infection risk, providing a rationale to inform the vaccination schedule for future efficacy trials.

RV305 (ClinicalTrials.gov NCT01435135) is a randomized, double blind, placebo-controlled trial of late boosts in RV144 vaccine recipients using ALVAC-HIV or AIDSVAX B/E, either combined or alone. The primary objectives were to evaluate cellular and humoral responses in systemic and mucosal compartments after late boosts and assess safety and tolerability.

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[a]S. R. N. and P. Pitisuttithum contributed equally to this work.

[b]S. V. and R. J. O. contributed equally to this work.

[c]Present affiliation: International Vaccine Initiative, Seoul, Republic of Korea.

[d]Present affiliation: Tripler Army Medical Center, Honolulu, Hawaii.

[e]Present affiliation: NeoImmune Tech, Rockville, Maryland.

[f]Study team members are listed in the Acknowledgments.

Correspondence: R. J. O'Connell, MD, Department of Retrovirology, Armed Forces Research Institute for Medical Sciences (AFRIMS), Bangkok, Thailand (oconnellrj@hiv-th.org).

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METHODS

Volunteers

Volunteer subjects were healthy, HIV-uninfected Thai RV144 vaccine recipients. Female participants agreed to use contraception for 45 days before the first and 3 months after the final vaccination. All volunteers provided written informed consent. The study was approved by ethical review boards at the Walter Reed Army Institute of Research, the Thai Ministry of Public Health, the Royal Thai Army Medical Department, the Faculty of Tropical Medicine, Mahidol University, Chulalongkorn University Faculty of Medicine, and Siriraj Hospital.

Vaccines

ALVAC-HIV (vCP1521) is a recombinant canarypox vector vaccine expressing CRF01_AE HIV-1 glycoprotein (gp) 120 (92TH023) linked to the transmembrane-anchoring portion of subtype B gp41 (strain LAI) with a deletion in the immunodominant region also expressing HIV-1 Gag and protease (strain LAI). A new production lot of the identical product administered in RV144 was used in this study (manufactured by IDT Biologika for Sanofi Pasteur) [6]. The vaccine was formulated, reconstituted, and administered into the left deltoid muscle as in the RV144 trial [6].

AIDSVAX B/E vaccine used in RV144 [6] was manufactured by Genentech for Global Solutions for Infectious Diseases (formerly VaxGen) as a bivalent HIV-1 gp120 glycoprotein composed of 300 μg of subtype B (MN) and 300 μg of CRF01_AE (A244) proteins adsorbed onto a total of 0.6 mg of aluminum hydroxide gel. AIDSVAX placebo was manufactured by Hollister-Stier for Global Solutions for Infectious Diseases as a suspension of 0.6 mL of aluminum hydroxide. This study administered doses of AIDSVAX B/E vaccine and placebo from identical lots as RV144, administered as a 1.0-mL intramuscular injection into the right deltoid muscle.

Study Design

Volunteers were randomized into groups and to receive vaccine or placebo at a ratio of 45:9 per group in a blinded manner. Group 1 received ALVAC-HIV and AIDSVAX B/E, group 2 received AIDSVAX B/E, and group 3 received ALVAC-HIV, or placebo, at weeks 0 and 24 (Figure 1).

Volunteers recorded local and systemic reactions on a diary card for 3 days after vaccination. Adverse events (AEs) occurring up to 3 months after last vaccination and all serious AEs (SAEs) throughout the trial were recorded. Safety laboratory assessments including urine dipstick, complete blood cell count with differential, plasma creatinine level, and liver enzyme values were obtained at baseline and week 72. Female participants underwent urine pregnancy testing at baseline, immediately before each vaccination and/or optional invasive procedures, and at study completion.

HIV Diagnostic Methods

HIV infection status was determined at screening and at weeks 0, 24, 48, and 72 using Genetic Systems HIV-1/HIV-2 Plus O Enzyme Immunoassay (EIA; Bio-Rad Laboratories) and Genetic Systems HIV-1 Western Blot (Bio-Rad Laboratories). Reactive EIA samples were repeated in duplicate to confirm reactivity before Western blot testing, which was interpreted as positive in the presence of ≥2 of the major bands, gp160 and/or gp120, gp41, and p24. All positive or indeterminate blot samples underwent nucleic acid testing using Amplicor HIV-1 Monitor test, version 1.5 (Roche Molecular Systems) to confirm HIV infection.

HIV-1 Env-Specific Plasma IgG Binding Antibody

HIV-1-specific plasma IgG enzyme-linked immunosorbent assay antibody responses were assessed using rgp120 and scaffold proteins performed, as described elsewhere [13]. Capture

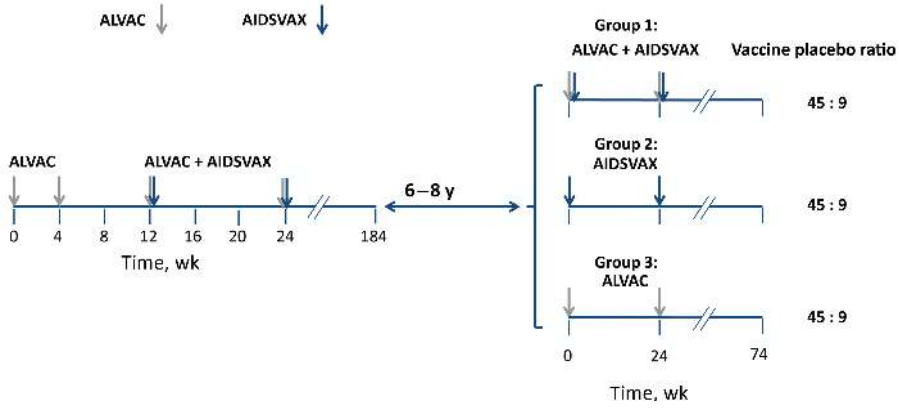

Figure 1. RV305 study design. *Left,* RV144 study vaccinations. Each RV305 participant received ALVAC-HIV (abbreviated ALVAC) and AIDSVAX B/E (abbreviated AIDSVAX) at the indicated time points, followed by a 6–8-year interval before RV305 enrollment. Participants were randomized to 1 of 3 groups and received inoculations at weeks 0 and 24. Group 1 received both ALVAC and AIDSVAX, group 2 received AIDSVAX alone, and group 3 received ALVAC alone. Participants were randomized within each group to receive either active vaccine product or corresponding placebo injections in a 5:1 ratio, and followed up for 12 months after the last injection.

antigens were chosen either because they represented vaccine sequences (A244gD and MNgD gp120 antigens identical to the AIDSVAX B/E proteins) or because they were correlates of risk in RV144 (gp70V1V2 92TH023 [12] and gp70V1V2 case A2 [8]).

Neutralizing Antibody Assessment

Neutralizing antibodies were measured as a function of reductions in luciferase (*luc*) reporter gene expression after a single round of infection in TZM-bl cells, as described elsewhere [20]. Stocks of molecularly cloned Env-pseudotyped viruses were prepared by transfection in 293T/17 cells (American Type Culture Collection) and titrated in TZM-bl cells as described elsewhere [20]. This assay has been formally optimized and validated [21] and was performed in compliance with Good Clinical Laboratory Practices, including participation in a formal proficiency testing program [22]. Tier 2 neutralization was assessed using a panel of 11 CRF01_AE pseudoviruses, and a global panel, as described elsewhere [23, 24].

Plasma IgA Binding Antibody

Env-specific IgA responses were longitudinally profiled in a subset of plasma samples randomly selected from each vaccine group at RV144 and RV305 baseline and peak immunogenicity time points against a panel of multiclade vaccine strain and consensus HIV-1 Env and V1V2 and V3 antigens by binding antibody multiplex assay, as described elsewhere [8, 25–27]. Postselection data checks identified no significant differences in age or sex distribution among the 3 vaccine groups. Assays were performed at a 1:40 final dilution of IgG-depleted plasma samples. The following antigens (provided by H. Liao and B. Haynes, Duke University) were assayed: group M consensus gp120 Env protein Con6 gp1202; ConSgp140; clade B Env protein MN gp120 gDneg/293T3; clade A proteins 92Th023 gp120 gDneg 293F/monomer4, A1.con.env03 140 CF4, 00MSA 4076 gp1404, clade AE proteins A244 gp120 gDneg delta 11/293T/mon4, HV13700 AE.con.env03 140 CF; V1V2 scaffold proteins gp70_B.caseA_V1_V2 and gp70_B.caseA2 V1V2/169K; and V3 protein B.MN V3 gp70.

Intracellular Cytokine Staining

Intracellular cytokine staining (ICS) was performed as described elsewhere [28]. Cells were stimulated with pools of peptides for LAI gag (122 peptides; pepMixGag LAI; batch 020513SASS-1), 92TH023 Env (166 peptides; pepMixEnvTH023; batch 020513SASS-2) (both manufactured by JPT Peptide Technologies), and an HIV Env V2 pool (pool of 6 × 15 mers from Chiang Mai double recombinant Env sequence produced by JPT), or with positive control phorbol myristate acetate (1 μg/mL)/ionomycin (1 μg/mL) (both from Sigma-Aldrich) or with dimethyl sulfoxide–containing medium alone. Stimulations were performed in the presence of co-stimulatory molecules CD28/CD49d, CD107a phycoerythrin (PE)–cyanine 7, and CD154 PE–cyanine 5 (all from BD Biosciences), for 4 hours. Surface staining consisted of CD14, CD19, and CD56 BV510 (BioLegend) and CD4 Qdot605 (Life Technologies) for 30 minutes. Intracellular staining was performed with CD8 PerCP-eF710 and interferon (IFN) γ eFluor 450 (eBiosciences) and CD3 allophycocyanin-H7, interleukin 2 (IL-2) PE, interleukin 4 allophycocyanin, granzyme B AF700, and tumor necrosis factor α fluorescein isothiocyanate (all from BD Biosciences). Cells were washed and analyzed on a 4 laser FACS LSRII SORP cytometer (BD Biosciences).

Statistical Analysis

The study was powered (80% power and unadjusted 5% α value) to detect effect sizes of 30% in vaccine response rates and 0.6 standard deviation differences in mean assay levels between active arms. A 5:1 active-placebo ratio was selected to provide adequate numbers in the grouped placebo (n = 27) for safety and immunogenicity comparisons, as well as blinding for study assessments. Differences in frequency of safety outcomes between each group were assessed using either Fisher exact test or Pearson χ^2 test, as appropriate. Enzyme-linked immunosorbent assay titers were expressed as the reciprocal of the highest dilution that yielded an absorbance value of 405 nm >2.5 times the background value, absorbance value >0.25 (wells without proteins). Geometric mean titers (GMTs) were calculated with associated 95% confidence intervals. Neutralizing antibody response was considered positive for a titer >20. ICS analysis excluded samples with <5000 $CD4^+$ or $CD8^+$ T cells acquired. Positivity was determined via comparison to match unstimulated versus stimulated responses, using Fisher exact test with Bonferroni adjustment.

Statistical comparisons between groups were assessed using Mann–Whitney *U* tests. Comparisons between time points were assessed using Wilcoxon signed rank tests; Differences were considered statistically significant at $P < .05$. To assess a change in ICS response rates after vaccination, an exact version of McNemar's test was used. To assess polyfunctional T-cell responses, generalized estimating equations were used to estimate the mean COMPASS (Combinatorial Polyfunctionality Analysis of Single Cells) functionality score [14] or each stimulation and T-cell subset over 3 time points (visits 2, 3 and 5 using an autoregressive working correlation structure. Adjusted *Q* values were created using the false discovery rate method of Benjamini and Hochberg [29]. Wald tests for a difference in estimated mean score between each active treatment group and the pooled placebo groups at visits 3 and 5 were performed using Huber-White standard error estimates. Data analysis and graphs were generated using GraphPad Prism version 6.05 for Windows (GraphPad Software), SPSS version 2.0 (IBM), R version 2.15.1, and SAS 9.4 software.

RESULTS

Study Population

Screening of 261 individuals was conducted to satisfy planned enrollment of 162 volunteers (Supplementary Figure 1). The 2 most common reasons for medical screening failures were abnormal laboratory results (n = 30), and hypertension (n = 18). Of study participants, 94 (58%) were male; the mean age was 31.6 years (range, 25–39 years). A mean of 7.2 years (range, 6.0–8.3 years) had elapsed since the last RV144 vaccination. This interval did not differ between study groups or between vaccine and placebo recipients (Table 1). Of 162 volunteers receiving the first vaccination, 161 (99.4%) received both doses, with 1 withdrawal due to relocation. Three additional volunteers withdrew after the second vaccination but before study completion (2 receiving ALVAC-HIV and 1 receiving AIDSVAX B/E placebo).

Safety and Reactogenicity

SAEs were reported in 7 (4.3%) volunteers: 2, 2, 1, and 2 in groups 1, 2, and 3 and placebo recipients, respectively. The proportion of volunteers experiencing an SAE did not differ between vaccine groups compared with pooled placebo volunteers (P = .63, .63, and .55 for groups 1, 2, and 3, respectively). SAEs included 1 instance each of drug hypersensitivity, subcutaneous abscess (knee), motorcycle accident, influenza infection, automobile accident, anaphylaxis due to hornet sting, and acute sinusitis, none of which were product related. All resolved without sequelae. The proportion of volunteers experiencing an AE in each treatment group did not differ significantly from that in the placebo group (P = .62, .13, and .23 for groups 1, 2, and 3, respectively), or within each system organ class. Three pregnancies (all vaccine recipients after vaccination) were reported, with uncomplicated deliveries of healthy infants.

Local reactions occurred after either or both doses in 96%, 71%, and 78% in groups 1, 2, and 3, compared with 55% in the placebo group (P = .01). Systemic reactions were observed significantly more frequently in groups 1 (78%; P < .001) and 3 (67%; P = .008) than in the placebo group (33%). Additional details may be found in the supplementary online material.

Vaccine-Induced Seroreactivity

No volunteers were EIA reactive at study entry. Of 134 vaccine recipients, 12 (8.9%) became EIA reactive at least once, including 7 (5.2%) at the end of the study. At 6 months after the first and second boosts, group 1 had similar EIA reactivity rate (3 of 45; [6.7%] and 4 of 45 [8.9%], respectively) compared with group 3 (5 of 44 [11.4%] and 8 of 44 [18.2%], respectively). Similarly, EIA reactivity in group 1 (4 of 45; 8.9%) was similar to that in group 3 (3 of 44; 6.8%) at the end-of-study time point. No vaccine recipients from group 2 had EIA reactivity at any time point. No significant differences in EIA reactivity rate between groups were found at any time point. All 27 samples with EIA reactivity produced an indeterminate Western blot with 100% band reactivity at p24, p40, p51, and p55, and 0% reactivity at p18, p31, p41, p65, gp120, and gp160. HIV-1 RNA was below the limit of detection (<50 copies/mL) in these 27 participants.

Binding Antibody

Binding antibody results are shown in Figure 2. There were no significant differences between ALVAC-HIV/AIDSVAX B/E and AIDSVAX B/E groups for any comparisons. As compared with the peak immunogenicity time point in RV144 (week 26), both ALVAC-HIV/AIDSVAX B/E and AIDSVAX B/E groups yielded significantly higher IgG GMT against all capture antigens at weeks 2 and 26. ALVAC-HIV/AIDSVAX B/E group A244 gp120 titers at these 2 time points rose significantly from 10 383 to 67 986 (P < .001). Although the post–second boost titer of 19 279 was significantly higher than the RV144 peak (P < .001), it remained lower than the peak after first RV305 vaccination (P < .001).

A similar pattern was observed for both responses against gp70 V1V2 (92TH023), with titers of 999, 14 069, and 3052 (P < .001 both for pairwise comparisons between RV144 titers and each RV305 peak titer and for the comparison between RV305 weeks

Table 1. Demographic Characteristics of the RV305 Study Population

	Group 1	Group 2	Group 3		
Characteristic	ALVAC-HIV+ AIDSVAX B/E	AIDSVAX B/E	ALVAC-HIV	Pooled Placebo Recipients[a]	Total
Sex, total participants (%)					
Male	23 (51.1)	26 (57.8)	24 (53.3)	21 (77.8)	94 (58)
Female	22 (48.9)	19 (42.2)	21 (46.7)	6 (22.2)	68 (42)
Age, y					
Mean (SD)	32.5 (3.7)	31.4 (3.3)	31.2 (3.8)	31.2 (3.1)	31.6 (3.5)
Range	(25–38)	(25–39)	(25–38)	(26–36)	(25–39)
Time since last RV144 dose, y					
Mean (SD)	7.3 (0.5)	7.1 (0.5)	7.2 (0.6)	7.1 (0.6)	7.2 (0.6)
Range	(6–8.1)	(6–7.9)	(6–8.3)	(6–8.3)	(6–8.3)

Abbreviation: SD, standard deviation.

[a]Data analyzed were combined from RV144 placebo recipients across all RV305 groups.

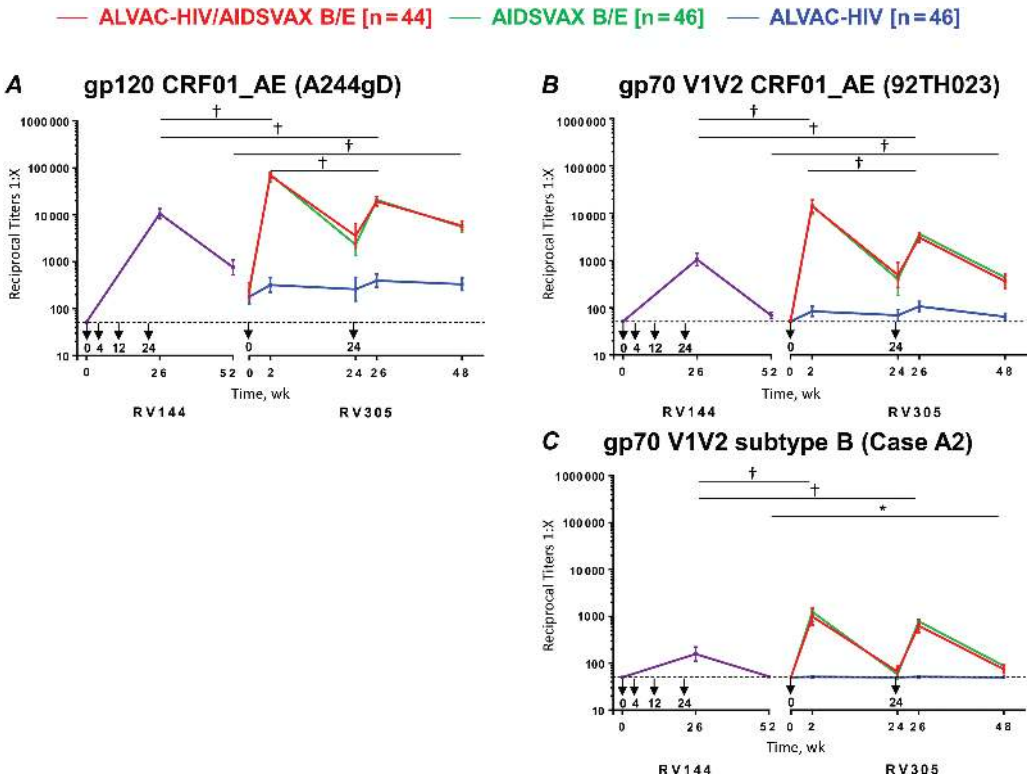

Figure 2. Immunoglobulin (Ig) G binding antibody responses against gp120 and scaffolded variable regions 1 and 2 (V1V2) antigens. Reciprocal titers against gp120 (A244 gD (*A*), gp70 V1V2 (92TH023) (*B*), and gp70 V1V2 (case A2) (*C*) are shown. Each panel graphically depicts titers for RV305 on the right, and for these same individuals in RV144 on the left, as along with numeric depiction of geometric mean titers, color coded where red represents ALVAC-HIV/AIDSVAX B/E; green, AIDSVAX B/E; blue, ALVAC-HIV; and purple, RV144. Error bars depict 95% confidence intervals. RV305 vaccine administration timing is indicated by black arrows. By study design, all volunteers were RV144 active product recipients, completing the 4 vaccination series on RV144 study week 24 (vaccinations not shown). Statistical significance was assessed using the Mann–Whitney *U* test. *P* values are color coded for pairwise within-group comparisons between time points indicated by black bars. Only comparisons reaching statistical significance at the level of $P < .05$ are shown. Results from RV305 volunteers randomized to receive placebo did not differ significantly from those from the ALVAC-HIV group (data not shown). $^{*}P < .05$ to .001; $^{\dagger}P < .001$.

2 and 26), and against gp70 V1V2 (case A2), with titers of 148 982 and 625 ($P < .001$ for comparisons between RV144 titer and RV305 weeks 2 and 26; $P = .13$ for comparison between RV305 weeks 2 and 26). ALVAC-HIV did not induce significant titers against any of the capture antigens (difference not significant for all comparisons with RV305 placebo recipients). When responses were evaluated as the percentage of samples with a positive response (Supplementary Table 1), differences between RV144 and RV305 time points were less apparent in groups 1 and 2, which produced near-universal response rates against gp120 A244 gD and gp70 V1V2 92TH023, but responses to gp70 V1V2 case A2 showed a similar pattern to that observed when data were expressed using GMTs. Compared with 6 months after final RV144 vaccination, RV305 vaccinations did not improve the durability of antibody responses against any capture antigens. RV144 fold-decreases against gp120 (A244gD), gp70 V1V2 (92TH023), and gp70 V1V2 (case A2) were 14, 14, and 3, compared with 19, 29, and 15 at RV305 weeks 2 and 26.

Neutralizing Activity

At baseline in RV305 week 0), weak positive neutralizing activity was detected against MN.3 (63 of 70; 90%; all groups combined), MW965.26 (45 of 70; 64.3%) and to a lesser degree TH023.6 (11 of 70; 15.7%) as an indication of long-lived Env-specific B-cell responses generated by previous RV144 immunizations (Figure 3). No neutralization of SF162.LS was detected at baseline. After the first boost, neutralizing antibody titers rose dramatically against all 4 viruses in groups 1 and 2, exceeding peak titers in RV144. Substantial titers were again seen against all 4 viruses in groups 1 and 2 after the second boost, of lower magnitude than after the first boost but significantly higher than peak titers in RV144 ($P < .001$ by Wilcoxon rank sum test). No significant difference was seen between groups 1 and 2 for any virus at either time point. Little or no detectable increase in neutralizing activity was seen in groups 3 or placebo after either boost. Rare, weak tier 2 virus neutralization activities were observed (data not shown).

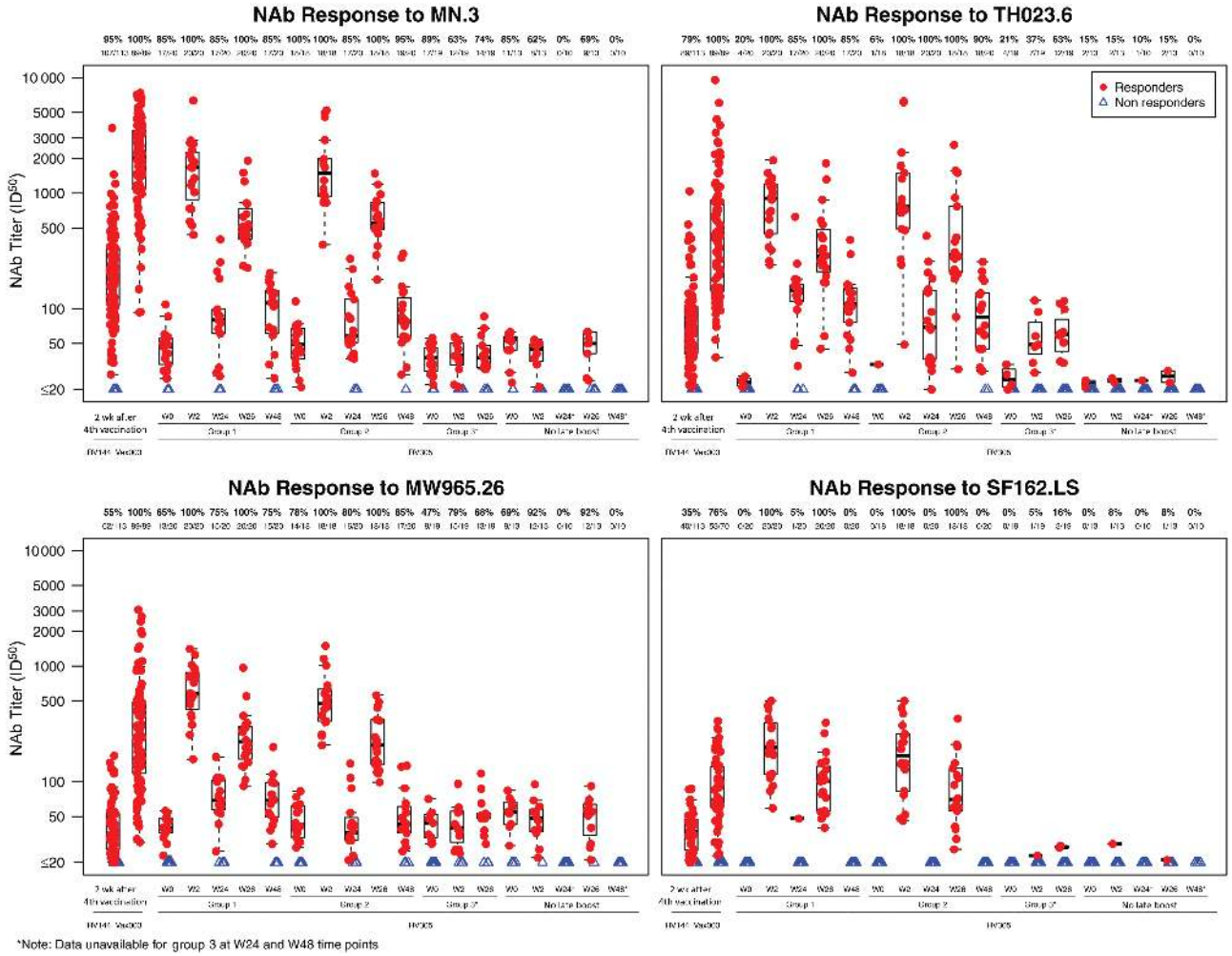

Figure 3. Neutralizing antibody (Nab) activity against tier 1A envelope (Env)–pseudotyped viruses assayed in TZM-bl cells. Neutralization was assessed before the first boost (week 0 [W0]), 2 weeks after the first boost (W2), and 2 weeks after the second boost (W26). Assays were performed with 2 of the vaccine strains (MN.3, protein boost; TH023, vCP1521 prime) and 2 heterologous tier 1A viruses (MW965.26, clade C; SF162.LS, clade B). RV305 placebo recipients are designated as "no late boost" volunteers to emphasize that although they received placebo during RV305, they are not vaccine naive owing to previous receipt of RV144 vaccines. Medians are shown with 95% confidence interval with median, and boxes represent interquartile ranges. ID_{50}, 50% inhibitory dose.

IgA-Binding Antibody Responses

IgA responses to 6 HIV-1 Env proteins and 2 V1V2 scaffolds were measured in serial plasma samples collected from 32 randomly selected volunteers (12, 8, and 12 volunteers from groups 1, 2, and 3, respectively) (Figure 4). There was no significant difference between response magnitudes to any analyte between groups 1 and 2 at RV305 weeks 2 and 26 (Wilcoxon exact test, $P > .05$), or in response rate by Fisher exact test (Supplementary Table 2). There was no significant difference in response rate between the 2 groups. (Not significant was defined as $P > .05$ for all comparisons where the response rates were other than 100%; no difference, response rate of 100% for both groups).

To determine whether Env IgA was significantly increased after RV305 immunizations compared with the peak immunogenicity time point in RV144, data from groups 1 and 2 were combined (n = 20), because there was no evidence of significant difference between these groups. Group 3 (ALVAC-HIV) was not considered for this analysis, because for most of the analyte-week combinations the response magnitude was low or below the linear range of detection of the assay. The first RV305 immunization significantly increased gp120 and gp140 plasma IgA binding responses at weeks 2 and 26, over the RV144 peak immunogenicity at week 26 (sign test with false discovery rate correction, $P < .05$).

Cellular Immune Responses

The proportion of positive $CD4^+$ T-cell IFN-γ responses against HIV-1 Env protein (92TH023) was significantly higher at week 2 than at week 0 for group 1 ($P = .002$), and at both weeks 2 and 26 for group 2 ($P = .03$ for both) (Figure 5A). Week 2 IL-2 responses were less frequent, with a significantly higher proportion of responses than at week 0 only in group

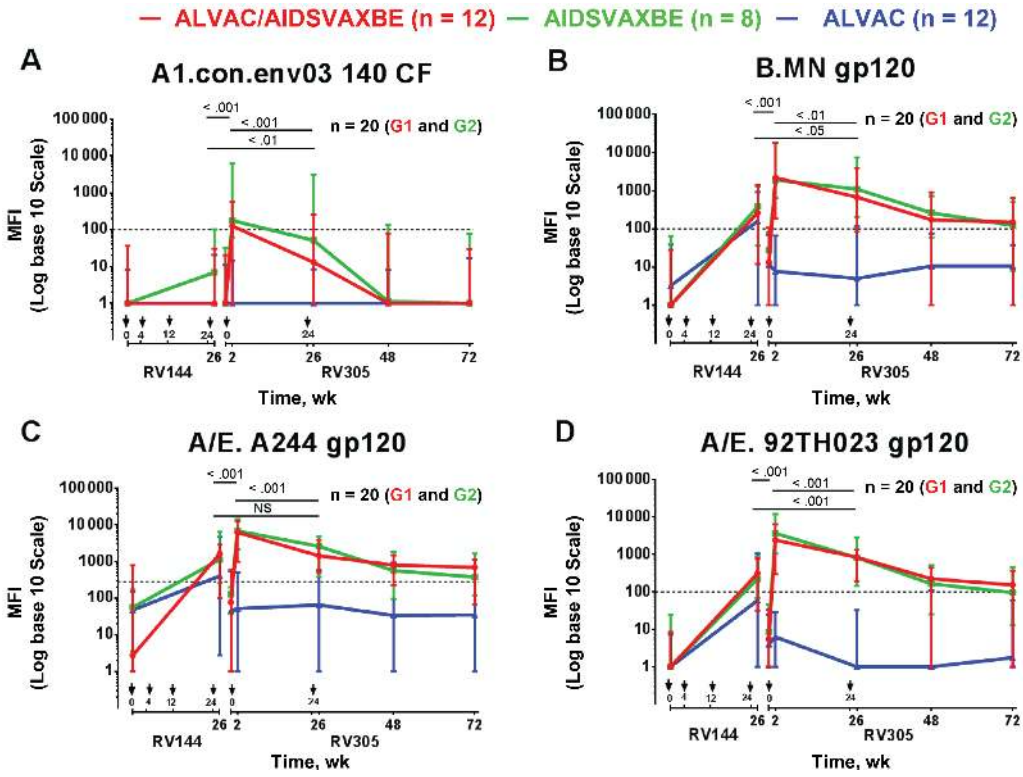

Figure 4. Immunoglobulin A antibody responses to human immunodeficiency virus (HIV) type 1 envelopes induced in RV144 and RV305. Binding antibody multiplex assay responses to A1.con gp140 (*A*), B.MN gp120 (*B*), A/E.A244 gp120 (*C*), and A/E.92TH023 gp120 (*D*). The sample sizes were 12, 8, and 12 for groups 1, 2, and 3, respectively. Medians and ranges are shown. The threshold for positivity is shown by a dashed line and is calculated for each envelope protein separately, as the mean + 3 standard deviations (after removing outliers >5 times the interquartile range) of the mean fluorescent intensity (MFI) from ≥30 RV144 baseline plasma samples assayed at 1:40 dilution, or a minimum of 100.

2 (P = .008) (Figure 5B). Response magnitudes ranged from 0.3% to 0.4% (IFN-γ), and from 0.07% to 0.3% (IL-2) (data not shown). $CD4^+$ T-cell responses against V2 loop peptides or HIV Gag peptides and $CD8^+$ T-cell responses to all peptides were negligible (data not shown). For $CD4^+$ T-cell functionality scores, 6 comparisons between the active treatment groups and the pooled placebo group were significant ($P \leq .046$) (Figure 5C).

These results demonstrate an increased $CD4^+$ functionality with HIV-1 Env peptides (92TH023) stimulation in groups 1 and 2 at 2 weeks after the first and second vaccinations. The estimated mean functionality score was greatest in group 1, followed by group 2 (mean, 0.10 vs 0.08) at 2 weeks after the first boost (visit 3). In both groups the functionality score was lower 2 weeks after the second boost (mean, 0.058 vs 0.058) but still elevated compared with the pooled placebo group (mean, 0.03; $P < .001$ for both comparisons). None of the comparisons were significant for $CD8^+$ T-cell functionality scores (Q values ≥ 0.26).

DISCUSSION

Both AEs and local/systemic reactogenicity occurred significantly more frequently in vaccine recipients compared with placebo, similar to observations in RV144 [30]. Weak residual HIV-specific responses from RV144 were present at the beginning of RV305, and these responses rose dramatically after additional boosting with AIDSVAX B/E. Boosting in groups 1 and 2 produced significantly higher Env-specific plasma binding antibody titers that were inverse correlates of risk in RV144 [8, 12], compared with titers measured 2 weeks after the last RV144 vaccination. Such responses did not differ between groups 1 and 2, demonstrating no measurable contribution by ALVAC-HIV on antibody induction at these time points.

These observations demonstrate that memory responses to the RV144 vaccination regimen persisted, suggesting that late boosting with AIDSVAX B/E with or without ALVAC-HIV might be a strategy to overcome suboptimal efficacy induced by the RV144 regimen. Unfortunately, late boosts failed to increase durability of antibody responses. Finally, regimens for both groups 1 and 2 induced neutralizing antibody responses with higher magnitude against tier 1 viruses compared with RV144, but they failed to induce tier 2 responses. Recent characterizations of antibodies identified from a subpopulation of B-cell lineages from groups 1 and 2 volunteers suggest that late boosts induce longer HCDR3 and higher rates of somatic hypermutation compared with RV144. Although these populations remain subdominant,

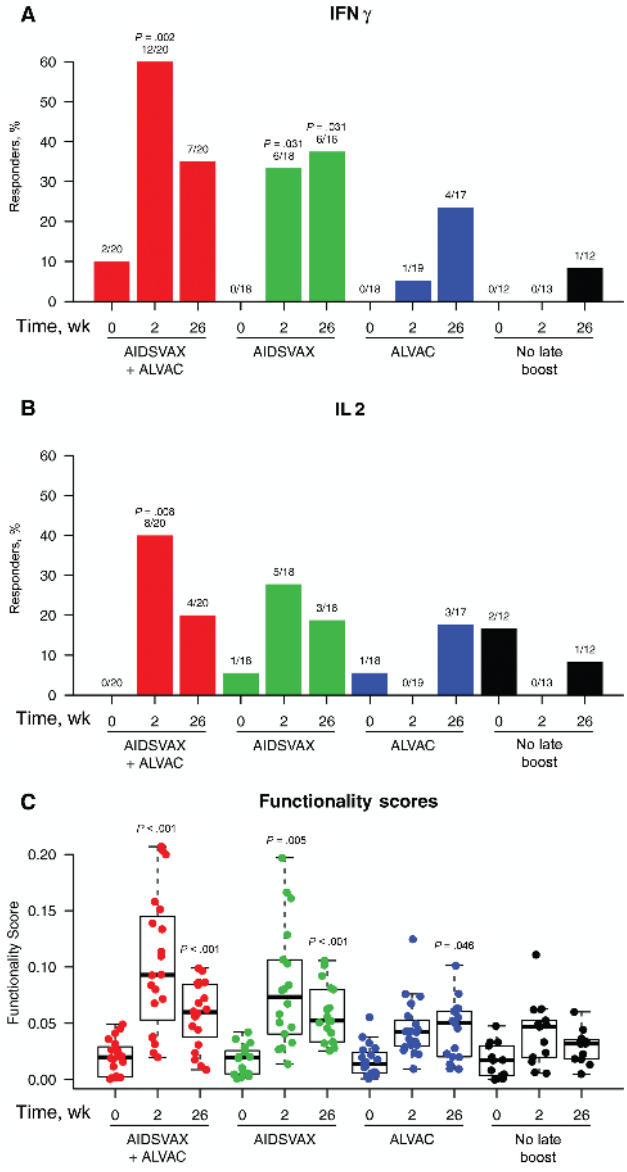

Figure 5. CD4+ T-cell responses. *A, B,* Percentage of vaccine responders as measured by interferon (IFN) γ (*A*), or interleukin-2 (IL-2) (*B*) production at study weeks shown on the x-axis for each study group; group 1 (ALVAC-HIV + AIDSVAX B/E) is shown in red, group 2 (AIDSVAX B/E) in green, group 3 (ALVAC-HIV) in blue, and volunteers (who did not receive active vaccine during this late boost study) in black. RV305 placebo recipients are designated as "no late boost" volunteers to emphasize that although they received placebo during RV305, they are not vaccine naive owing to previous receipt of RV144 vaccines. *C,* COMPASS (Combinatorial Polyfunctionality Analysis of Single Cells) functionality score by treatment group and visit for T cells stimulated with human immunodeficiency virus (HIV) type 1 envelope protein (92TH023), based on the 6 cytokines measured (154, 107, IL-2, interleukin 4 [IL-4], IFN-γ, and tumor necrosis factor [TNF] α). Points show the observed functionality score, and box plots show the median and interquartile range. Whiskers extend out to the most extreme data point that is ≤1.5 times the interquartile range from the upper or lower quartile. Functionality scores were compared between active treatment groups and the pooled RV305 placebo recipients at weeks 2 and 26, using a Wald test as described in Methods. Significant *P* values with a corresponding multiplicity adjusted *Q* value ≤ 0.2 (method of Benjamini and Hochberg) are shown above the corresponding box plot. The median and range of the mean fluorescence intensity are plotted by RV305 vaccine regimen (G1 ALVAC-HIV/AIDSVAX B/E in red, G2 AIDSVAX B/E in green, and G3 ALVAC-HIV in blue), for the 4 antigens A1.con gp140, B.MN gp120, A/E.A244 gp120, and A/E.92TH023 gp120, by week tested (bold font below x-axis). Medians from adjacent weeks are connected by lines.

the changing characteristics demonstrate that key features similar to some V1V2-targeting broadly cross-neutralizing antibodies are induced by this strategy [31].

Vaccination in groups 1 and 2 induced both binding and tier 1 virus neutralizing antibody responses that were highest after the first RV305 vaccination (6–8 years after the last vaccination) but lower after the second study vaccination (6 months after the last vaccination). The high response after a long rest interval suggests an anamnestic response related to B-cell memory induced by previous vaccinations. Alternatively, lower response after a shorter rest interval may be related to either B-cell exhaustion [32] or immune tolerance [33] and may be influenced by adaptive T-cell responses or innate immune responses that prevent optimal boosting. Studies to further elucidate these mechanisms are ongoing.

Booster vaccinations with ALVAC-HIV alone induced minimal cellular responses and negligible humoral responses, suggesting limited value as a stand-alone late boosting agent. Results from ongoing analyses. including IgG subclasses, antibody-dependent cellular cytotoxicity, functional B-cell and innate cellular responses, are required for a final assessment between late boosts with AIDSVAX B/E alone versus the combination with ALVAC-HIV.

The rest interval from last RV144 vaccination to first RV305 injection was much longer than ideal for a regimen employed to maintain rapidly waning antibody responses. A recently completed study, RV306 (ClinicalTrials.gov NCT01931358) vaccinated HIV vaccine-naive adults in Thailand receiving the RV144 regimen plus ALVAC-HIV/AIDSVAX B/E or AIDSVAX B/E boosts at week 48, or ALVAC-HIV / AIDSVAX B/E at week 60 or 72, to assess rest intervals of 24, 36, and 48 weeks between the fourth and fifth vaccinations. HVTN 100 is a trial of similar products with HIV subtype C inserts and MF59 adjuvant in South Africa with a fifth vaccination at month 12 after the first immunization. Immunogenicity assessments for both trials are ongoing, and will inform late boost schedules and help elucidate determinants of immunologic memory.

Boosting RV144 vaccinees 6–8 years later was safe and well tolerated. Regimens containing AIDSVAX B/E with or without ALVAC-HIV generated increased, but short-lived, humoral and CD4+ T-cell responses that did not rise further after subsequent boosting. Additional studies of immune responses in anatomic locations relevant to transmission, alternative vaccine schedules, and use of more potent adjuvants are needed to better characterize the utility of delayed boosting intervals as strategies to improve and extend protective responses arising from preventive HIV vaccines.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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