Antibody Responses in Anogenital Secretions of RV305, a Late Boost Vaccination of RV144 Volunteers

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Abstract

Anogenital mucosae are the primary sites of HIV-1 acquisition. RV144 is the only HIV vaccine trial to show efficacy to date. HIV uninfected RV144 volunteers (n=162) who completed all vaccinations 6-8 years earlier, were enrolled into the RV305 and were randomized to receive 2 injections of ALVAC-HIV or AIDSVAX®B/E or a combination of both, or placebo, at day 0 and 6 months. We measured serial antibody responses in anogenital secretions.

IgG and IgA responses in cervico-vaginal mucus (CVM), seminal plasma (SP), and rectal secretion (RS) at study entry and 2 weeks post first and second injection were tested by ELISA against HIV-1 envelope gp120 CRFo1_AE (A2449D), and gp70V1V2 scaffold proteins derived from CRFo1_AE (92TH023) and subtype B (CaseA2).

Prior to boosting, IgG A2449D responses in CVM were similar in all vaccination groups with geometric mean titers (GMT) range of 61-66. GMT increased to 1745 and to 904 two weeks after the first boost, but decreased to 994 (p=0.10) following the second boost in AIDSVAX®B/E and ALVAC-HIV/AIDSVAX®B/E groups, respectively. Gp70V1V2 IgG responses in CVM were undetected in all groups prior to boosting. After the first injection, responses were higher in AIDSVAX®B/E followed by ALVAC-HIV/AIDSVAX®B/E; gp70V1V2 B GMT=35 and 28, respectively; gp70V1V2 AE, GMT=228 and 133, respectively. After the second boost, responses to gp70V1V2 AE decreased to GMT=65 (p<0.01) and 50 (p=0.04), respectively, but responses to gp70V1V2 B remained unchanged. In SP, IgG responses to A2449D and gp70V1V2 AE were detected after the first boost in the AIDSVAX®B/E and ALVAC-HIV/AIDSVAX®B/E groups with A2449D GMT=125 and 126, respectively, and for gp70V1V2 AE GMT=28 in both groups. IgG GMT to A2449D decreased to 62 (p<0.01) and 70 (p<0.01), respectively, while gp70V1V2 AE reactivity was undetectable after the second boost. SP IgG responses to gp70V1V2 B were not detected in either group. In the ALVAC-HIV group, CVM and SP IgG responses were undetectable for all proteins tested. IgG responses were not detected in RS from any vaccination group and no IgA responses were detected in any of the samples tested (1:100 sample dilution).

Antibodies induced in RV305 anogenital secretions indicate that IgG responses to HIV-1 gp120 and to gp70V1V2 scaffold proteins in CVM and SP were inducible after being boosted with AIDSVAX®B/E or ALVAC-HIV/AIDSVAX®B/E. We did not detect IgG antibody responses in mucosal secretions from the group receiving ALVAC-HIV only and in RS from all groups. IgA responses were absent in mucosal secretions from all groups. The induction of HIV-1 antigen specific IgGs in CVM and SP suggests a possible mechanism by which the RV144 regimen provided protection from HIV-1 acquisition in heterosexual couples.

Background

RV144, a community-based, randomized, double blind, placebo-controlled phase III efficacy trial of the prime-boost combination of vaccines containing ALVAC-HIV (vCP1521) and AIDSVAX®B/E conducted in Rayong and Chonburi provinces of Thailand, demonstrated a modest protective efficacy of 31.2% against HIV-1 acquisition (i). Two immune response variables significantly associated with HIV-1 infection risk among RV144 study participants: plasma IgG binding antibodies to HIV-1 gp120 variable regions 1 and 2 (V1/V2) envelope (Env) proteins inversely correlated with risk, while plasma monomeric IgA binding antibodies to viral Env directly correlated with risk (ii) and may be associated with HIV-1 acquisition (iii-iv). Further studies of immune responses in plasma of vaccine recipients as well as identification of a sieve effect identified in the V2 region (v) reinforce the importance of immune responses directed against V1/V2 region.

Anogenital mucosae are the primary site of HIV-1 acquisition (vi). Mucosal HIV-1 specific antibodies are thought to be involved in protection of viral acquisition at mucosal sites (vii). Half of the antibodies in genital secretions originate from peripheral blood, while most antibodies in intestinal secretions are produced by plasma cells present in the lamina propria (viii). This raises the hypothesis that intramuscular immunization with HIV-1 antigens may induce HIV-1 specific antibodies in genital secretions but not in the intestinal tract. However, immune responses in mucosal secretions of RV144 participants remain uncharacterized.

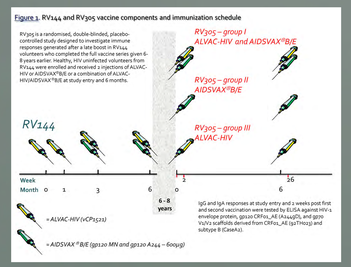
To investigate whether antibody responses are present in anogenital secretions after systemic immunization, we measured serial mucosal secretions from HIV-1 uninfected subjects who received the entire active RV144 vaccine regimen 6-8 years before receipt of two additional booster vaccinations 6 months apart.

Methods

Study design

A total of 162 healthy HIV-uninfected vaccine recipients who completed the full vaccination series from RV144 were randomized to receive one of three booster or placebo regimens in a follow-up protocol (RV305). Each group included 45 vaccine and 9 placebo recipients who were immunized at study entry and 6 months. Group I received ALVAC-HIV/AIDSVAX®B/E or ALVAC placebo/AIDSVAX placebo, group II received AIDSVAX®B/E or AIDSVAX placebo, and group III received ALVAC-HIV or ALVAC placebo. The protocol received approval from local and international Institutional Review Boards. Written informed consent was obtained from all volunteers. Figure 1 shows the vaccine components and immunization schedule. Anogenital samples from consenting volunteers who completed week 26 (2 weeks post second boost) were assessed blinded to immunization status.

Figure 1. RV144 and RV305 vaccine components and immunization schedule



Methods (cont.)

Vaccines

ALVAC-HIV (vCP1521) is a recombinant canarypox vector vaccine expressing Env gp120 CRF01_AE (92TH023) linked to the transmembrane-anchoring portion of gp41 subtype B (LAI), and gag and protease subtype B (LAI), administered intramuscularly at the dose of 2.06 $5 CCID50 (Sanofi Pasteur).

AIDSVAX®B/E is a bivalent vaccine of gp120 subtype B (MN) and gp120 CRF01_AE (A244) adsorbed into aluminium hydroxide gel adjuvant and administered intramuscularly at a dose of 600µg (300µg of each antigen; Global Solutions for Infectious Diseases - GSID).

Anogenital samples

Cervico-vaginal mucus (CVM), seminal plasma (SP), and rectal secretions (RS) were collected using Instead Softcup (Evofem, San Diego, CA), masturbation, and Merocel schindler ear packing sponges (Medtronic Xomed, Jacksonville, FL), respectively. Samples were tested at study entry time-point and 2 weeks post first and second injections. All participants were HIV-1 uninfected at the time of specimen collection.

Reagents and assays

Recombinant gp120 CRF01_AE (A2449D) protein, gp70V1V2 CRF01_AE (92TH023) and gp70V1V2 Subtype B (CaseA2) scaffolds were used as antigens for assessing IgG and IgA responses by antibody binding ELISA assays. ELISA for all antigens was performed using U-bottom 2HB plates coated with 3 µg/mL of recombinant gp120 or gp70 in D-PBS at 4°C overnight. Wells were washed 3 times with wash buffer (PBS, 0.1% Tween20 and 0.01% Thimerosal, pH 7.4) using Microplate Washer ELx405 (BioTek, Winooski, VT) and blocked with 220 µL/well of blocking buffer (D-PBS, 5% skim milk) for 2 hours at room temperature. Anogenital samples were initially diluted 1:100 (responses to gp120) or 1:25 (responses to gp70) in blocking buffer and two-fold serial dilutions were performed, and then 100 µL of diluted specimen was added to wells for a 2-hour incubation at room temperature. All wells were washed 5 times with wash buffer and 100 µL/well of HRP conjugated goat anti-human IgG, IgA, at 1:25000 dilution, was added and incubated for 1 hour at room temperature. Plates were washed 5 times with wash buffer, 100 µL/well of substrate was added, and color was allowed to develop at room temperature for 1 hour in the dark. Plates were read at 405nm absorbance (Spectramax 340 PC ELISA reader, Molecular Devices, Downingtown, PA).

Total concentration of IgG and IgA antibodies in anogential secretions was measured to ensure antibody integrity. Samples testing positive for blood were not included in the analysis. Study was blinded and placebos were included in the analysis.

Discussion

IgG responses against HIV-1 gp120 and to gp70V1V2 scaffold proteins in CVM and SP were detected following booster vaccination with AIDSVAX®B/E or ALVAC-HIV/AIDSVAX®B/E but not with ALVAC-HIV, in volunteers previously vaccinated with the complete RV144 ALVAC-HIV/AIDSVAX®B/E regimen. Responses two weeks post first vaccination were higher than those 2 weeks post second vaccination for gp120 and gp70V1V2 CRF01_AE for both CVM and SP but weak antibody responses to gp70V1V2 subtype B were the same 2 weeks post first and second vaccination in CVM and absent from SP. This finding argues against boosting at 6 month intervals as a strategy to increase the magnitude of responses. Responses to the gp70V1V2 CRF01_AE were higher than those induced by gp70V1V2 subtype B, a finding that is consistent with plasma responses to scaffold antigens that have been demonstrated to be correlates of risk in post-RV144 analyses (ix). The most likely explanation of this difference is stronger induction of homologous anti-V1/V2 responses by the CRF01_AE Env immunogens used in this vaccine regimen compared to the subtype B immunogens. IgG antibody responses were not detected in RS suggesting that this regimen may not induce strong antibody responses in the gastrointestinal tract. Another possible explanation is insufficient antibody detection due to methodologic considerations, but detection of total Ig antibodies argues against this possibility. IgA responses to all tested proteins were undetectable in anogenital secretions from all groups at the plasma concentrations tested. This study is limited by studying only baseline and two-week post injection time-points. Analysis of additional inter-injection and late time-points will allow for more complete characterization of antibody durability.

Conclusions

The induction of gp120 and V1/V2 loop specific IgGs in CVM and SP suggests a possible mechanism by which the RV144 regimen could have provided protection from HIV-1 acquisition in heterosexual couples.

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Disclaimer

The opinions herein are those of the authors and should not be construed as official or representing the views of Mahidol University, Ministry of Public Health, the U.S. Department of Health and Human Services, National Institute of Allergy and Infectious Diseases, the Department of Defense or the Department of the Army.

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Results

At study entry, IgG responses to gp120 A2449D in CVM were similar in all vaccination groups with geometric mean titers (GMT) ranging between 61 and 66. The magnitude of responses varied markedly among volunteers but overall significantly increased to 1745 (p<0.01) and to 904 (p<0.01) two weeks after the first injection, but decreased to 994 (p=0.01) and 556 (p=0.10) following the second injection in AIDSVAX®B/E and ALVAC-HIV/AIDSVAX®B/E groups, respectively (**Figure 2**). IgG responses to both gp70V1V2 92TH023 and gp70V1V2 CaseA2 in CVM were undetected in all groups at study entry. After the first injection, responses were higher in AIDSVAX®B/E followed by ALVAC-HIV/AIDSVAX®B/E group; gp70V1V2 92TH023, GMT=228 and 133, respectively (**Figure 3**); gp70V1V2 CaseA2, GMT=35 and 28, respectively (**Figure 4**). After the second boost, responses to gp70V1/V2 92TH023 significantly decreased to GMT=65 (p<0.01) and 50 (p=0.04), respectively (**Figure 3**), and demonstrated distinct subgroups of high- and low-responders, but responses to gp70V1V2 CaseA2 remained unchanged (**Figure 4**).

In SP, IgG responses to gp120 A2449D and gp70V1V2 92TH023 were detected after the first injection in the AIDSVAX®B/E and ALVAC-HIV/AIDSVAX®B/E groups with gp120 A2449D GMT=125 and 126, respectively (**Figure 5**), and for gp70V1V2 92TH023 GMT=28 in both groups (**Figure 6**). IgG responses to gp120 A2449D significantly decreased to 62 (p<0.01) and 70 (p<0.01), respectively (**Figure 5**), while gp70V1V2 92TH023 reactivity was reduced (GMT=14) after the second injection (**Figure 6**). IgG responses to gp70V1V2 CaseA2 in SP were not detected in either group (**Figure 7**). In the ALVAC-HIV group, IgG responses to all proteins tested were undetectable in CVM and SP (**Figures 2-7**).

IgG antibody responses to antigens tested were not detected in RS from any vaccination group (data not shown) and IgA antibody responses were undetectable in any of the samples tested (1:100 sample dilution), (data not shown).

Figure 2. IgG ELISA binding antibody responses to gp120 A2449D envelope protein in RV305 Cervico-Vaginal Mucus (CVM)

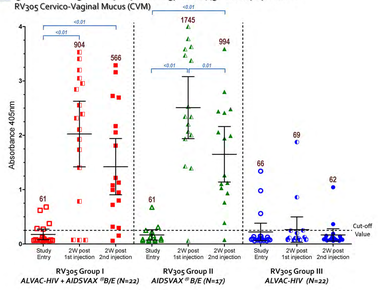

Figure 3. IgG ELISA binding antibody responses to gp70V1V2 92TH023 scaffold in RV305 Cervico-Vaginal Mucus (CVM)

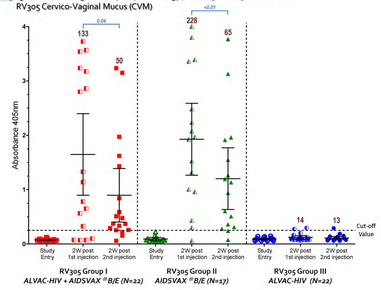

Figure 4. IgG ELISA binding antibody responses to gp70V1V2 CaseA2 scaffold in RV305 Cervico-Vaginal Mucus (CVM)

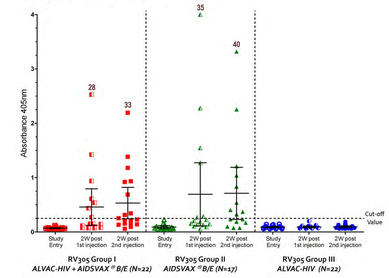

Figure 5. IgG ELISA binding antibody responses to gp120 A2449D envelope protein in RV305 Seminal Plasma (SP)

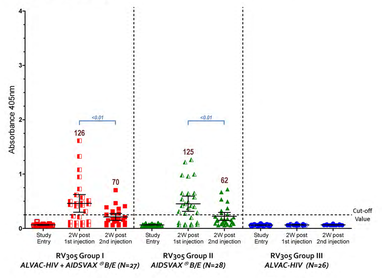

Figure 6. IgG ELISA binding antibody responses to gp70V1V2 92TH023 scaffold in RV305 Seminal Plasma (SP)

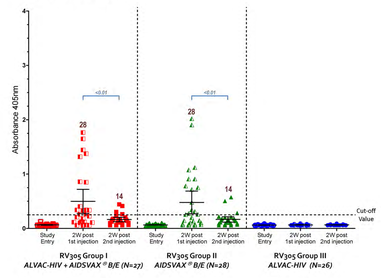

Figure 7. IgG ELISA binding antibody responses to gp70V1V2 CaseA2 scaffold in RV305 Seminal Plasma (SP)

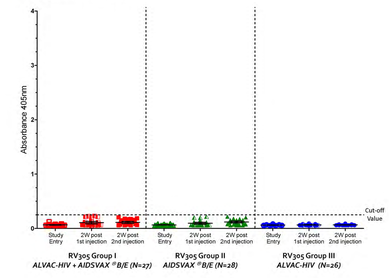

Figure Legends:
Bars are means of absorbance 405nm with 95% confidence intervals, numeric values on graphs depict geometric mean titers. "2W post 1st injection" and "2W post 2nd injection" indicate two weeks after receiving the first and second vaccination, respectively, ALVAC-HIV/AIDSVAX®B/E vaccine or ALVAC/AIDSVAX placebo in RV305 group I regimen; AIDSVAX®B/E vaccine or AIDSVAX placebo in RV305 group II regimen; ALVAC-HIV vaccine or ALVAC placebo in RV305 group III regimen. The study is blinded and placebos are included in graphs.