AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 31, Number 11, 2015
DOI: 10.1089/aid.2015.0034

IgG Antibody Responses to Recombinant gp120 Proteins, gp70V1/V2 Scaffolds, and a CyclicV2 Peptide in Thai Phase I/II Vaccine Trials Using Different Vaccine Regimens

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Abstract

RV144 correlates of risk analysis showed that IgG antibodies to gp70V1V2 scaffolds inversely correlated with risk of HIV acquisition. We investigated IgG antibody responses in RV135 and RV132, two ALVAC-HIV prime-boost vaccine trials conducted in Thailand prior to RV144. Both trials used ALVAC-HIV (vCP1521) at 0, 1, 3, and 6 months and HIV-1 gp120MNgD and gp120A244gD in alum (RV135) or gp120SF2 and gp120CM235 in MF59 (RV132) at 3 and 6 months. We assessed ELISA binding antibodies to the envelope proteins (Env) 92TH023, A244gD and MNgD, cyclicV2, and gp70V1V2 CaseA2 (subtype B) and 92TH023 (subtype CRF01_AE), and Env-specific IgG1 and IgG3. Antibody responses to gp120 A244gD, MNgD, and gp70V1V2 92TH023 scaffold were significantly higher in RV135 than in RV132. Antibodies to gp70V1V2 CaseA2 were detected only in RV135 vaccine recipients and IgG1 and IgG3 antibody responses to A244gD were significantly higher in RV135. IgG binding to gp70V1V2 CaseA2 and CRF01_AE scaffolds was higher with the AIDSVAX®B/E boost but both trials showed similar rates of antibody decline post-vaccination. MF59 did not result in higher IgG antibody responses compared to alum with the antigens tested. However, notable differences in the structure of the recombinant proteins and dosage used for immunizations may have contributed to the magnitude and specificity of IgG induced by the two trials.

Introduction

The Thai "Phase III" trial, RV144, showed an estimated vaccine efficacy of 31.2% at 42 months, and post hoc analysis suggested that efficacy at 12 months was 60% (95% CI 2–80%).[1,2] The vaccine regimen consisted of a nonreplicating recombinant canarypox vector, ALVAC-HIV (vCP1521) prime and AIDSVAX® gp120 B/E boost. The vaccine-induced plasma IgG binding antibody to scaffolded gp70V1V2 envelope proteins from multiple HIV-1 subtypes correlated inversely while high levels of Env plasma IgA (monomeric) binding score correlated directly with HIV

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acquisition.[3–5] Viral sieve analysis supported a role for the second variable domain of Env (V2) in protection.[6] Peptide microarray analysis from six HIV-1 subtypes and group M consensus showed that the vaccination regimen induced antibody responses to the V2 loop of gp120 of multiple subtypes. V2 responses by ELISA and surface plasmon resonance were further evaluated using cyclic (CycV2) and linear V2 loop peptides. Ninety-seven percent of volunteers had antibody responses against CycV2 at 2 weeks post-last immunization, declining to 19% 6 months later.[7]

Whether quantitative and qualitative antibody responses to soluble HIV-1 envelope (Env) protein subunits can be modulated by adjuvants remains a critical question for the selection of Env immunogens in future efficacy trials.[8,9] We investigated HIV-specific binding antibody responses to whole gp120 proteins, gp70V1V2 scaffolds, a CycV2 peptide, and IgG subclasses in two phase I/II prime-boost vaccine trials conducted in Thailand prior to RV144 (RV135[10] and RV132[11]). RV135 was the phase I/II forerunner to RV144 with the identical vaccine components and immunization regimen. Both trials used ALVAC-HIV (vCP1521) as a prime and each used a different bivalent HIV-1 gp120 protein boost formulated either in alum (RV135) or in MF59 (RV132) adjuvant.

Materials and Methods

Vaccines and immunization regimens

ALVAC-HIV (vCP1521) (Sanofi Pasteur, Marcy-l'Etoile, France) is a recombinant canarypox vector genetically engineered to express Env gp120 of the HIV-1 CRF01_AE 92TH023 strain linked to the transmembrane anchoring portion of subtype B gp41 (with a deletion in the immunodominant region devoid of the entire gp41 ectodomain), and HIV-1 Gag and protease (both LAI strain). ALVAC-HIV (vCP1521) was administered at a dose of $10^{6.5}$ $CCID_{50}$. AIDSVAX® B/E vaccine (Global Solutions for Infectious Diseases, GSID, South San Francisco, CA) used in both RV144 and RV135 is composed of gp120 HIV-1 subtype B MN and HIV-1 gp120 CRF01_AE A244, each containing a 27 amino acid (aa) sequence from the herpes simplex virus gD protein fused to each protein at the N-terminus. MNgD and A244gD gp120 proteins were expressed in CHO cells, adsorbed onto aluminum hydroxide gel adjuvant, and combined to produce the bivalent AIDSVAX® B/E vaccine administered at 600 μg (300 μg of each rgp120).[1,10,12] Bivalent gp120 B/CRF01_AE vaccine used in RV132 was also produced in CHO cells (Novartis Vaccines and Diagnostics, Cambridge, MA) and contained 100 μg of gp120 from the CRF01_AE strain CM235 and 50 μg from the subtype B strain SF2, formulated in MF59 adjuvant.[11] Both trials used the same immunization schedule used in RV144, with administration of ALVAC-HIV at 0, 1, 3, and 6 months and gp120 protein boosts at 3 and 6 months.

Specimens and study subjects

Plasma samples from 15 vaccine and 6 placebo recipients (RV132) and 30 vaccine and 10 placebo recipients (RV135) were randomly selected. Both studies had received approval of appropriate Institutional Review Boards and written informed consent was obtained from all volunteers. Samples were tested at baseline, 2 weeks post-second ALVAC vaccination, 2 weeks post-third and fourth vaccinations (protein boosts), and 6 months post-fourth vaccination. All participants were HIV-1 uninfected at the time of blood draw. All plasma and serum specimens were stored at −80°C.

Recombinant proteins and CycV2 peptide

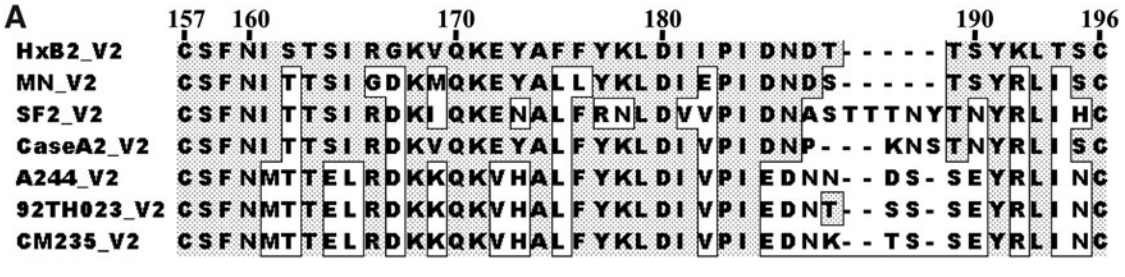
Recombinant gp120 CRF01_AE (A244gD and 92TH023) and subtype B (MNgD) were expressed in 293T cells and purified on *Galanthous nivalis* lectin columns.[7] Scaffold gp70V1V2 proteins (subtype B CaseA2 and CRF01_AE 92TH023) were expressed and purified as described previously.[5,13] The CycV2 peptide was synthesized by JPT Peptide Technologies (Acton, MA). V2 peptides were cyclized by disulfide bond formation with a purity >90% measured by high-pressure liquid chromatography and mass spectrometry. Amino acid sequences of the CycV2 peptide were based on Env glycoprotein 92TH023. Strain 92TH023, CM235, and A244 V2 loops vary by two amino acids at positions 188 and 189 (HXB2 numbering) but the antibody binding mid-region is identical[7] (Fig. 1A and B). Therefore, CycV2 peptides from A244 and CM235 were not included in the study. The CycV2 peptide contained 42 aa extending from aa 158 to 199 (corresponding to HIV-1 HXB2 aa 157–196). Percent identity of Env gp120 used in the vaccines is shown in Fig. 1C.

ELISA for recombinant gp120 proteins, gp70V1V2 scaffolds, and CycV2 peptide

As described previously,[7] ELISA for rgp120, gp70 V1V2 scaffolds, and the CycV2 peptide was performed using U-bottom 2HB plates coated with either 1 μg/ml of a cyclic peptide or with 3 μg/ml of the recombinant gp120/gp70 in D-PBS (Sigma-Aldrich, St. Louis, MO) at 4°C overnight. Wells were washed three times with wash buffer (PBS, 0.1% Tween 20, and 0.01% Thimerosal, pH 7.4, Sigma-Aldrich, St. Louis, MO) using Microplate Washer ELX405 (Bio Tek, Winooski, VT), and blocked with blocking buffer (D-PBS, 5% skim milk, Applichem, St. Louis, MO) for 2 h at room temperature. Plasma was initially diluted in blocking buffer and serial 2-fold dilutions were performed and added to wells for 2 h at room temperature. Wells were washed with wash buffer and HRP-conjugated goat antihuman IgG at 1:25,000 dilution was added and incubated for 1 h at room temperature. Plates were washed, ABTS ELISA HRP substrate (KPL, Gaithersburg, MD) was added, and color was allowed to develop at room temperature for 1 h in the dark. Plates were read at A_{405} nm using an ELISA reader Spectramax 340 PC (Molecular Devices, Sunnyvale, CA). For IgG subclasses (IgG1 and IgG3) binding, plates were coated with antigen as in regular ELISA and plasma was initially diluted 1:25 in blocking buffer and serial 2-fold dilutions were performed and added to wells for 1-h incubation at room temperature. Wells were washed and mouse antihuman IgG1 or IgG3 (Invitrogen, Grand Island, NY) was added for an hour at room temperature. Plates were washed and HRP-conjugated goat antimouse IgG (Southern Biotech, Birmingham, AL) was added and incubated for 1 h at room temperature. Plates were washed; substrate was added and then read as described above.

Statistical methods

ELISA antibody titers were calculated using serial 2-fold dilutions of plasma from 1:100 to 1:12,800 and expressed as



B Percent Identity of V2 Loops

	HxB2	MN	SF2	CaseA2	A244	92TH023	CM235
HxB2	100%	75%	62.5%	79.5%	52.5%	55.0%	52.5%
MN	75%	100%	72.5%	84.6%	60.0%	60.0%	60.0%
SF2	62.5%	72.5%	100%	78.6%	57.1%	57.1%	59.5%
CaseA2	79.5%	84.6%	78.6%	100%	65.9%	65.9%	65.9%
A244	52.5%	60%	57.1%	65.9%	100%	95.2%	95.2%
92TH023	55.0%	60.0%	57.1%	65.9%	95.2%	100%	95.2%
CM235	52.5%	60.0%	59.5%	65.9%	95.2%	95.2%	100%

C Percent Identity of gp120 ENVs

	HxB2	MN	SF2	A244	92TH023	CM235
HxB2	100%	83.0%	84.6%	69.6%	70.1%	70.5%
MN	83.0%	100%	83.3%	71.0%	70.8%	72.3%
SF2	84.6%	83.3%	100%	71.5%	72.1%	73.6%
A244	69.6%	71.0%	71.5%	100%	93.1%	94.2%
92TH023	70.1%	70.8%	72.1%	93.1%	100%	95.2%
CM235	70.5%	72.3%	73.6%	94.2%	95.2%	100%

FIG. 1. Alignment and percent identity of V2 loops and percent identity of HIV-1 gp120 envelopes. **(A)** *Shaded sequences* match HxB2 and *boxed* residues differ from the HxB2 reference strain. Amino acid sequences of the V2 loop are numbered based on the HxB2 reference strain. **(B)** Percent identity of the V2 loops. **(C)** Percent identity of gp120 Envs. RV132: HIV-1 gp120 92TH023 (CRF01_AE in ALVAC-HIV), CM235 (CRF01_AE), and SF2 (subtype B). RV135: HIV-1 gp120 92TH023 (CRF01_AE in ALVAC-HIV), A244 (CRF01_AE), and MN (subtype B).

the reciprocal of the highest dilution that yielded an absorbance value above 2.5 times the background value. An overall false-positive response was calculated for each protein and peptide, stratified by clinical trial based on the 95th percentile from all baseline absorbance data of vaccine recipients. Antibody responses to an individual protein or peptide were expressed as percentage of subjects with a positive response, defined as A_{405} nM absorbance value >0.25 (positive response rate).

Nonparametric inferential statistical methods were used throughout to analyze antibody titers that were non-normally distributed (data not shown). Geometric mean titers (GMT) were calculated with associated 95% confidence intervals (95% CI). Mean fold-change (visit 7/visit 5 titers) was calculated between receipt of the first and second subunit vaccine doses and its associated *p*-value using the Wilcoxon matched pairs method. Comparisons between groups (RV132 and RV135) were performed using the Mann–Whitney test using the Benjamini–Hochberg method to control the false discovery rate.[14] Statistical analyses were performed with Graphpad Prism 6.0 (GraphPad Software, San Diego, CA), Stata SE 11 (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX), and R (R Core Team 2013; R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, www.R-project.org/).

Results

ELISA IgG antibody responses to rgp120 proteins

HIV-specific IgG responses were not detected at baseline, in placebo, and post-second ALVAC-HIV administration in both regimens (data not shown). Table 1 and Figs. 2A–C and 3A–C show the results of the analysis of IgG binding antibody responses. A majority of subjects demonstrated responses 2 weeks post-first (V5) and post-second (V7) gp120 protein boosts (Fig. 2A–C). gp120-specific binding antibody was still present 6 months after protein administration (V10) in both RV135 and RV132 vaccine recipients. However, these responses were significantly lower than those at peak immunogenicity (visit 7). Antibody GMT to gp120 A244gD, the homologous antigen for RV135, was significantly higher in RV135 than in RV132 post-first (4422 vs. 606, $p = 0.0137$) and post-second (9050 vs. 2425, $p = 0.0002$) boosts and 6 months post-second boost (1240 vs. 159, $p = 0.0002$), respectively (Fig. 2A). GMT to the heterologous gp120 92TH023 did not differ significantly between RV135 and RV132 post-first boost (229 vs. 418, $p = 0.2439$), post-second boost (4422 vs. 3676, $p = 0.4662$), and 6 months post-second boost (209 vs. 191, $p = 0.8179$) (Fig. 2B). Antibody responses to homologous vaccine antigen gp120 MNgD were also significantly higher in RV135 than in RV132 at all time points: post first boost (4422 vs. 174, $p = 0.0002$), post second boost (19,855 vs. 1269, $p = 0.0002$), and 6 months post-second boost (1924 vs. 114, $p = 0.0002$), respectively (Fig. 2C). For both regimens, GMT decreased sharply 6 months post-second boost.

ELISA IgG antibody responses to CycV2 peptide and gp70V1V2 scaffolds

GMT to CycV2 92TH023 peptide did not differ significantly post-first protein boost (129 vs. 209, $p = 0.4662$) and post-second boost (310 vs. 332, $p = 0.9712$) between RV135 and RV132, respectively (Table 1 and Fig. 3A). Antibody binding to CycV2 MN and SF2 peptides was not tested. V2 antibodies were not detected in placebo recipients (data not shown).

Antibody GMT to gp70V1V2 CaseA2 scaffold (used in the RV144 correlates analysis and found to inversely correlate with HIV-1 acquisition risk) was significantly higher in RV135 than in RV132 (191, 77% vs. 52, 0%, respectively) (Table 1 and Fig. 3B). Antibody GMT to gp70V1V2 92TH023 scaffold was significantly higher post-second boost in RV135 than in RV132 (1131 vs. 481, $p = 0.0190$) (Table 1 and Fig. 3C). Six months post-boost, for both vaccine regimens, there was a sharp fall in frequency and titers to both scaffolds.

Table 1. Magnitude and Frequency of Binding Antibodies to HIV-1 rgp120 Envelope Proteins, a Cyclic V2 Peptide, and gp70V1V2 Scaffold Proteins in RV132 (ALVAC-HIV Prime with CRF01_AE CM235 and Subtype B SF2 Protein Boost) and RV135 (ALVAC-HIV Prime with AIDSVAX® B/E Protein Boost) Vaccine Recipients

		GMT (95% CI)		
Antigen	*Protocol*	*Visit 5 2 weeks post-first protein boost*	*Visit 7 2 weeks post-second protein boost*	*Visit 10 6 months post-second protein boost*
gp120A244gD	RV132 ($n=15$)	606 (258–1427)	2425 (1617–3637)	159 (106–237)
	No (% POS)	13 (87)	15 (100)	13 (87)
	RV135 ($n=30$)	4422 (1930–10131)	9050 (6853–11953)	1240 (823–1871)
	No (% POS)	29 (97)	30 (100)	30 (100)
	p-value[a]	0.0073	0.0001	0.0001
	p_c[b]	0.0137	0.0002	0.0002
gp120 92TH023	RV132	418 (181–970)	3676 (2640–5117)	191 (122–298)
	No (% POS)	11 (73)	15 (100)	13 (87)
	RV135	229 (147–359)	4422 (3321–5888)	209 (167–262)
	No (% POS)	23 (77)	30 (100)	29 (97)
	p-value	0.1626	0.3730	0.7634
	p_c	0.2439	0.4662	0.8179
gp120MNgD	RV132	174 (107–283)	1269 (828–1947)	114 (83–160)
	No (% POS)	12 (80)	15 (100)	11 (73)
	RV135	4222 (1875–9508)	19855 (14943–26381)	1924 (1296–2860)
	No (% POS)	28 (93)	30 (100)	30 (100)
	p-value	0.0001	0.0001	0.0001
	p_c	0.0002	0.0002	0.0002
CycV2 92TH023	RV132	209 (93–473)	332 (230–481)	NP
	No (% POS)	9 (60)	15 (100)	
	RV135	129 (90–185)	310 (206–468)	NP
	No (% POS)	17 (57)	26 (87)	
	p-value	0.3602	0.9712	
	p_c	0.4662	0.9712	
gp70V1V2 CaseA2	RV132	NP	52 (47–58)	NP
	No (% POS)		1 (7)	0 (0)
	RV135	NP	191 (133–274)	55 (50–60)
	No (% POS)		23 (77)	4 (13)
	p-value		0.0001	0.4701
	p_c		0.0002	0.5786
gp70V1V2 92TH023	RV132	NP	481 (283–819)	91 (66–126)
	No (% POS)		14 (93)	9 (60)
	RV135	NP	1131 (758–1689)	89 (67–119)
	No (% POS)		30 (97)	13 (43)
	p-value		0.0107	0.6105
	p_c		0.0190	0.6977
IgG1 gp120A244gD	RV132	NP	209 (134–327)	NP
	No (% POS)		15 (100)	
	RV135	NP	746 (558–999)	NP
	No (% POS)		30 (100)	
	p-value		0.0001	
	p_c		0.0006	
IgG1 gp70V1V2 CaseA2	RV132	NP	12.5 (12.5–12.5)	NP
	No (% POS)		0 (0)	
	RV135	NP	12.8 (12.2–13.4)	NP
	No (% POS)		1 (3)	
	p-value		0.8567	
	p_c		0.8567	
IgG1 gp70V1V2 92TH023	RV132	NP	52 (33–84)	NP
			14 (93)	
	RV135	NP	102 (66–158)	NP
	No (% POS)		28 (93)	
	p-value		0.0511	
	p_c		0.1022	

(*continued*)

TABLE 1. (CONTINUED)

Antigen	Protocol	GMT (95% CI) Visit 5 2 weeks post-first protein boost	GMT (95% CI) Visit 7 2 weeks post-second protein boost	GMT (95% CI) Visit 10 6 months post-second protein boost
IgG3 gp120A244gD	RV132	NP	87 (43–175)	NP
			14 (93)	
	RV135	NP	325 (221–478)	NP
	No (% POS)		30 (100)	
	p-value		0.0026	
	p_c		0.0078	
IgG3 gp70V1V2 Case A2	RV132	NP	12.5 (12.5–12.5)	NP
	No (% POS)		0 (0)	
	RV135	NP	12.8 (12.2–13.4)	NP
	No (% POS)		1 (3)	
	p-value		0.4795	
	p_c		0.5754	
IgG3 gp70V1V2 92TH023	RV132	NP	22 (12–40)	NP
	No (% POS)		4 (27)	
	RV135	NP	29 (21–40)	NP
	No (% POS)		19 (63)	
	p-value		0.0930	
	p_c		0.1395	

[a]*p*-value calculated based on Mann–Whitney *U* test.
[b]p_c, corrected *p*-value based on Mann–Whitney test adjusted for multiple corrections using the Benjamini–Hochberg method to control the false discovery rate.
GMT, geometric mean titer; CI, confidence interval; NP, not performed.

HIV antigen specificity of IgG subclasses

Two weeks post-second protein boost, IgG1 and IgG3 antibody responses to gp120 A244gD were significantly higher in RV135 where subjects were immunized with the homologous antigen than in RV132 (IgG1: 746 vs. 209, $p=0.0006$; IgG3: 325 vs. 87, $p=0.0078$, respectively) (Fig. 4). IgG1 antibodies to the heterologous gp70V1V2 92TH023 did not differ significantly between RV135 and RV132 (102 vs. 52, $p=0.1022$). IgG3 antibody responses

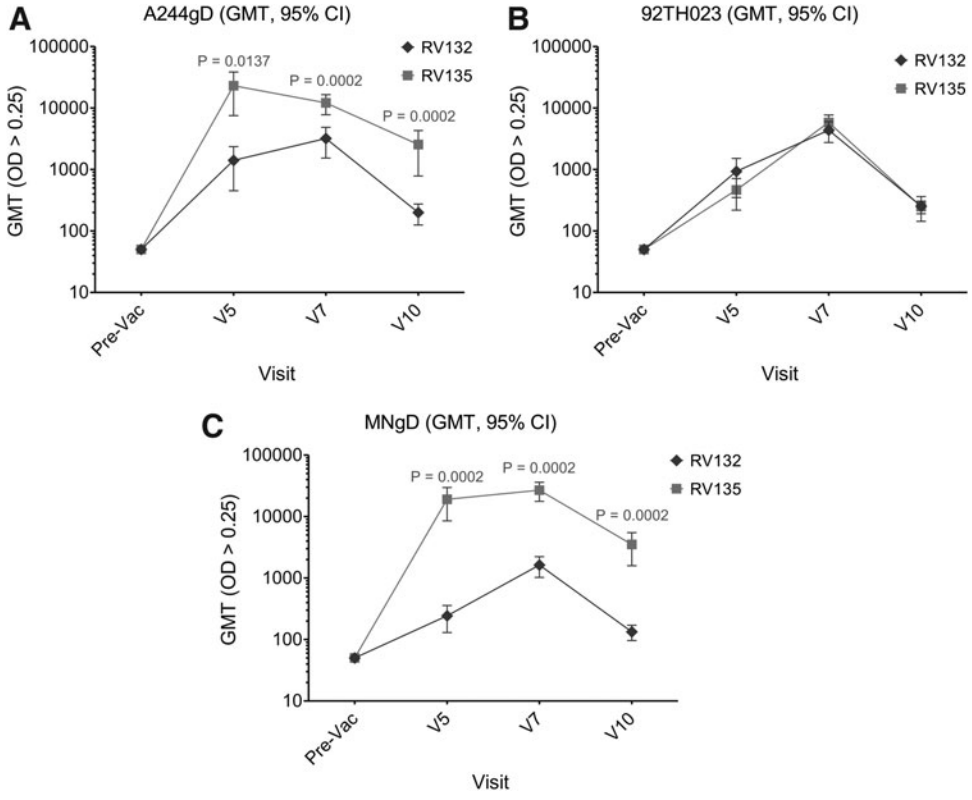


FIG. 2. Binding antibody geometric mean titers (GMT) to HIV-1 CRF01_AE rgp120 A244gD **(A)**, to 92TH023 **(B)**, and to HIV-1 subtype B MNgD **(C)** in RV135 (ALVAC-HIV prime with AIDSVAXB/E boost) and RV132 (ALVAC-HIV prime with CRF01_AE CM235 and subtype B SF2 boost) vaccine recipients. Pre-Vac: prevaccination; V5: 2 weeks post-first protein boost; V7: 2 weeks post-second protein boost; V10: 6 months post-second protein boost. Corrected *p*-value based on the Mann–Whitney test adjusted for multiple corrections using the Benjamini–Hochberg method.

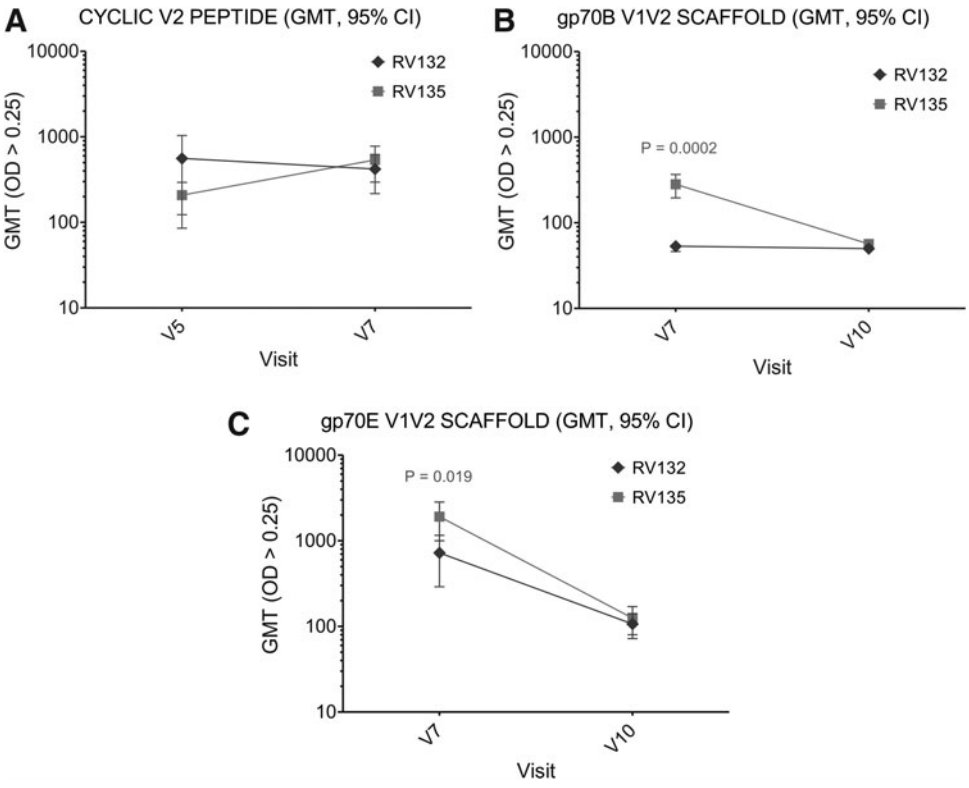


FIG. 3. Binding antibody geometric mean titers (GMT) to HIV-1 cyclic V2 peptide **(A)**, to HIV-1 gp70 V1V2 CaseA2 **(B)**, and to CRF01_AE 92TH023 **(C)** in RV135 (ALVAC-HIV prime with AIDSVAXB/E boost) and RV132 (ALVAC-HIV prime with CRF01_AE CM235 and subtype B SF2 boost) vaccine recipients. V5: 2 weeks post-first protein boost; V7: 2 weeks post-second protein boost; V10: 6 months post-second protein boost. Corrected *p*-value based on the Mann–Whitney test adjusted for multiple corrections using the Benjamini–Hochberg method.

were low and did not differ significantly (29 vs. 22, $p = 0.1395$) between the two trials. Low IgG1 and IgG3 binding antibody titers to gp70V1V2 CaseA2 were observed only in RV135 (Table 1).

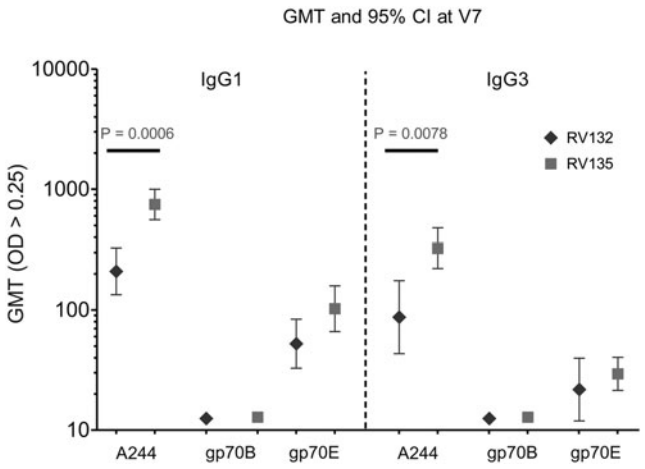


FIG. 4. IgG1 and IgG3 binding antibody geometric mean titers (GMT) to gp120 A244gD and scaffolded gp70 V1V2 proteins in RV135 (ALVAC-HIV prime with AIDSVAXB/E boost) and RV132 (ALVAC-HIV prime with CRF01_AE CM235 and subtype B SF2 boost) vaccine recipients. A244: HIV-1 CRF01_AE gp120 in AIDSVAX BE (RV135); gp70B: scaffolded gp70 V1V2 CaseA2 (subtype B); gp70E: scaffolded gp70 V1V2 92TH023 (CRF01_AE). V7: 2 weeks post-second protein boost. Corrected *p*-value based on the Mann–Whitney test adjusted for multiple corrections using the Benjamini–Hochberg method to control the false discovery rate.

Discussion

While RV135 and RV132 used the same ALVAC-HIV (vCP1521) prime, the bivalent gp120 B/E boost proteins, dose, and adjuvants differed. Bivalent gp120 AIDSVAX® B/E, tested in both VAX003[12] and RV144,[1] is composed of CRF01_AE A244gD and subtype B MNgD (300 μg of each rgp120), while in RV132, it is composed of CRF01_AE CM235 (100 μg) and subtype B SF2 (50 μg). Despite the differences in rgp120 B/E antigens, using a CRF01_AE (92TH023) heterologous to both vaccine strain gp120s allowed a comparison of gp120-specific titers. Both regimens elicited comparable levels of gp120-specific binding antibody to 92TH023 gp120. Antibody responses to MNgD and A244gD were higher in RV135.

Factors that may have contributed to antibody differences observed between the two trials include protein sequences, glycosylation patterns, dose of antigen (4-fold less in RV132), protein modifications, addition of a gD peptide, and a deletion of 11 amino acids (Δ11) at the N-terminus of gp120s in RV135, and adjuvants. Analysis using recombinant gp120 proteins showed that Δ11 modification without gD was sufficient to enhance responses to conformational epitopes on V1V2, V2, and other regions of the gp120. CM235 and SF2 share 94% and 83% amino acid identity with A244gD and MNgD, respectively.

Antibody responses to rgp120 CM235 and SF2 proteins were not evaluated due to sample/reagent constraints. In both RV132 and RV135, the binding antibody titers decreased significantly 6 months after the last protein boost indicating that both alum and MF59 did not sustain Env antibody responses 6 months post-second protein boost. The immunological mechanisms controlling antibody durability are not well understood but testing new immunogen/adjuvant

formulations that elicit broad and durable protection is needed for a successful vaccine.

Although we did not detect significant differences in antibody responses to 92TH023 (CRF01_AE) rgp120 protein and cyclic V2 peptide between the two vaccine trials, we observed differences in total IgG binding to the scaffold gp70V1V2 92TH023. Differences in antibody binding were also observed when the gp70V1V2 CaseA2 (heterologous to both vaccines) scaffold (subtype B) was used. The difference in binding could be attributed to the amino acid sequence in the recombinant proteins used in the vaccines.

Antibody responses to scaffold gp70V1V2 CaseA2 were detected only in RV135, which may suggest that qualitative differences in induced immune responses might be related to differences in either gp120 antigens, protein modifications, and/or adjuvants and dose of antigens. IgG responses from RV144 vaccinees to gp70V1V2 CaseA2[3,15] and other HIV-1 subtypes A, C, and CRF01_AE gp70V1V2 (92TH023) scaffold proteins were inversely correlated with risk, suggesting that this vaccine regimen might prevent acquisition of various HIV-1 clades.[5] However, antibody responses to other V1V2 scaffolds correlated with risk could not be tested in this comparative study. A recent study showed that RV144 linear IgG V2 responses were also associated with a lower risk of HIV-1 infection.[15]

We showed that IgG1 and IgG3 antibodies to A244gD were higher in RV135 than RV132 vaccinees. Previous studies with ALVAC-HIV prime (vCP1452) and alum-adjuvanted gp120 MNgD boost showed that antibody response were predominantly IgG1 with few weak IgG2 and IgG3 responses.[16] No significant differences were observed between the two regimens in IgG1 and IgG3 antibodies to gp70V1V2 92TH023 scaffold, although there was a trend for higher titers in RV135 that was significant when total IgG binding was measured. IgG1 and IgG3 antibody responses to gp70V1V2 CaseA2 scaffold were very weak and due to sample limitations we did not use lower sample dilutions to get a signal. It is unclear whether a higher concentration of recombinant proteins in the boost could have increased the magnitude of the antibody responses to gp70 CaseA2 scaffold in RV132. Chung *et al*. demonstrated that the RV144 regimen (identical to RV135) elicited nonneutralizing antibodies with highly coordinated Fc-mediated effector responses through the selective induction of highly functional IgG3 antibodies.[17] Analogous antibody responses might have been present in RV132 but were not assessed.

Alum is the most widely used vaccine adjuvant, but its mechanism of action remains largely unknown. MF59 is a safe and effective vaccine adjuvant that has been used in a licensed seasonal influenza vaccine for 15 years and is a stronger activator of cell recruitment than alum.[18] However, our study showed that in RV132 MF59 did not increase the magnitude, frequency, and durability of HIV-specific antibodies to the proteins and scaffolds tested. Whether higher concentrations of antigen and/or other recombinant proteins might have increased the magnitude, persistence, and quality of antibody responses in HIV-1 vaccine formulations that used MF59 remains unclear.

The lower protein doses chosen in RV132 vaccine represent an important variable to the current comparison, but dose sparing would likely be a key rationale for using MF59 in HIV vaccines as it has been in influenza vaccines.[19,20] A recent analysis of HIV-specific antibody responses in pediatric HIV vaccine trials PACTG 230[21,22] and PACTG 236[23] using recombinant clade B gp120 proteins (SF2, MN) in MF59 or ALVAC-HIV-1 (vCP1452) plus AIDSVAX® B′/B (MN/GNE8) in alum, respectively, indicated that in the MF59 gp120 trial, IgG responses to gp120 and gp70V1V2 CaseA2 were higher in magnitude and durability compared to the alum trial.[24]

The comparison between RV132, RV135, and the pediatric vaccine trials is difficult to evaluate because of fundamental differences in trial design: infants born to HIV-1-infected mothers, different immunization schedules and protein doses, and different ALVAC-HIV and AIDSVAX vaccines. In an NHP challenge study, immunizations with ALVAC-SIV and SIV gp120 in alum or MF59, only the alum group showed a significant reduction in SIV_{mac251} acquisition, while the MF59 did not despite its ability to elicit higher antibody responses. The frequency of plasmablasts expressing $\alpha_4\beta_7$ and CXCR4 (hematopoietic homing marker) was higher in the alum group, while there was a trend for a higher frequency of plasmablasts expressing CXCR3 (inflammatory site homing marker) in the MF59 group.[25] In other studies priming with alphavirus replicon particles encoding gp140ΔV2 and boosting with trimeric Env protein in MF59 adjuvant provided protection to macaques challenged intrarectally with $SHIV_{SF162P4}$.[26]

The relatively rapid decay of antibody responses observed in both trials has previously been reported in envelope protein alone and in prime-boost trials, whatever the envelope proteins and adjuvants used so far[1,10,11,27–30] with, however, a few exceptions.[24,31,32] Long-lived B cell memory represents the archive of antibody specificities that have occurred over much of the host lifespan.[33] In contrast, circulating antibodies usually decline after antigen clearance. The study of B cell memory and clonal exhaustion in future vaccine trials testing adjuvanted proteins might shed light on the mechanisms of sustainability of circulating antigen-specific antibodies.

Taken together, our results suggest that gp120 A244gD is qualitatively different from other gp120 proteins in inducing V2 antibody responses that bind to multiple subtypes.[34,35] Antibody titers to gp70V1V2 CaseA2 and to CRF01_AE gp70V1V2 were higher with the AIDSVAX® B/E boost, though both trials showed similar rates of antibody decline postvaccination suggesting that the formulations of these gp120 proteins at the doses tested with MF59 or alum did not translate with antibody persistence. Improved HIV-1 envelope antigens[34] formulated with more potent adjuvants[9] and/or more effective vaccine regimens are critically needed to induce stronger and more durable neutralizing and nonneutralizing functional antibodies.

Acknowledgments

MOPH-TAVEG Collaboration: Ministry of Public Health: Supachai Rerks-Ngarm, Prayura Kunasol, Nakorn Premsri, Chawetsan Namwat, Prasert Thongcharoen. Mahidol University: Punnee Pitisuttithum, Jaranit Kaewkungwal. Royal Thai Army: Sorachai Nitayaphan, Chirapa Eamsila. U.S. Military HIV Research Program: Nelson L. Michael, Jerome H. Kim, Robert J. O'Connell, Merlin L. Robb, Jean-Louis Excler, Robert Paris, Charla Andrews.

These studies were supported in part by an Interagency Agreement Y1-AI-2642-12 between the U.S. Army Medical

Research and Materiel Command (USAMRMC) and the National Institute of Allergy and Infectious Diseases. In addition, this work was supported by a cooperative agreement (W81XWH-07-2-0067) between the Henry M. Jackson Foundation for the Advancement of Military Medicine and the U.S. Department of Defense. This research was funded in part by the U.S. National Institute of Allergy and Infectious Diseases.

Preliminary results of this study were presented at the AIDS Vaccine Conference 2013, October 7–10, 2013, Barcelona, Spain (P03.27).

The opinions herein are those of the authors and should not be construed as official or representing the views of Mahidol University, the Thailand Ministry of Public Health, the Department of Defense, or the Department of the Army.

Author Disclosure Statement

James Tartaglia is an employee of Sanofi Pasteur, Faruk Sinangil and Donald P. Francis are employees of Global Solutions for Infectious Diseases, and Susan W. Barnett is an employee of Novartis Vaccines and Diagnostics, Inc. (now GlaxoSmithKline).

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