MAJOR ARTICLE

A Phase 1/2 Study of a Multiclade HIV-1 DNA Plasmid Prime and Recombinant Adenovirus Serotype 5 Boost Vaccine in HIV-Uninfected East Africans (RV 172)

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Background. Human immunodeficiency virus (HIV) vaccine development remains a global priority. We describe the safety and immunogenicity of a multiclade DNA vaccine prime with a replication-defective recombinant adenovirus serotype 5 (rAd5) boost.

Methods. The vaccine is a 6-plasmid mixture encoding HIV envelope (*env*) subtypes A, B, and C and subtype B *gag*, *pol*, and *nef*, and an rAd5 expressing identical genes, with the exception of *nef*. Three hundred and twenty-four participants were randomized to receive placebo ($n = 138$), a single dose of rAd5 at 10^{10} ($n = 24$) or 10^{11} particle units ($n = 24$), or DNA at 0, 1, and 2 months, followed by rAd5 at either 10^{10} ($n = 114$) or 10^{11} particle units ($n = 24$) boosting at 6 months. Participants were followed up for 24 weeks after the final vaccination.

Results. The vaccine was safe and well tolerated. HIV-specific T cell responses were detected in 63% of vaccinees. Titers of preexisting Ad5 neutralizing antibody did not affect the frequency and magnitude of T cell responses in prime-boost recipients but did affect the response rates in participants that received rAd5 alone ($P = .037$).

Conclusion. The DNA/rAd5 vaccination regimen was safe and induced HIV type 1 multi-clade T cell responses, which were not significantly affected by titers of preexisting rAd5 neutralizing antibody.

Trial Registration. ClinicalTrials.gov identifier: NCT00123968.

The human immunodeficiency virus (HIV) epidemic continues to grow, with 33 million people living with HIV/AIDS and an additional 7400 new infections daily in 2007 [1]. Although multiple HIV prevention strategies have been evaluated with some recent successes [2, 3], a vaccine to prevent HIV infection or alter disease course remains the cornerstone of a successful global HIV control program. Despite the failure of the VaxGen and Merck candidate HIV vaccines in efficacy testing [4–6], there is consensus that both basic discovery and clinical research pursuing a vaccine is justified [7]. A vaccine that induces primarily T cell immunity may only afford a reduction in viral burden among recipients on the basis of animal model studies and yet be a

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Table 1. Study Design

Group (no. of vaccinees/placebos)	Vaccination schedule			
	Day 0	Day 28	Day 56	Day 168
1 (24/12)	VRC-HIVADV014–00-VP, 10^{10} PU			
2 (24/12)	VRC-HIVADV014–00-VP, 10^{11}PU			
3 (24/12)	VRC-HIVDNA-016–00-VP, 4 mg	VRC-HIVDNA-016–00-VP, 4 mg	VRC-HIVDNA-016–00-VP, 4 mg	VRC-HIVADV014–00-VP, 10^{10} PU
4 (24/12)	VRC-HIVDNA-016–00-VP, 4 mg	VRC-HIVDNA-016–00-VP, 4 mg	VRC-HIVDNA-016–00-VP, 4 mg	VRC-HIVADV014–00-VP, 10^{11} PU
5 (90/90)	VRC-HIVDNA-016–00-VP, 4 mg	VRC-HIVDNA-016–00-VP, 4 mg	VRC-HIVDNA-016–00-VP, 4 mg	VRC-ADV014–00-VP, 10^{10} PU

NOTE. PU, particle unit.

valuable contribution to HIV control programs by slowing disease progression and reducing the rate of secondary infections [8, 9].

The Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), candidate HIV vaccine is a multiclade HIV-DNA prime vaccine expressing HIV type 1 (HIV-1) Gag, Pol, Nef, and Env proteins, boosted with recombinant adenovirus serotype 5 (rAd5) expressing matching proteins, with the exception of Nef. The VRC rAd5-DNA vaccine was designed for induction of HIV-specific T cell immunity, ideally to prevent HIV acquisition and, failing that, to reduce viral load in vaccine recipients who did become infected [10]. Unlike the Merck vaccine, which failed to provide benefit in efficacy testing, the VRC prime-boost candidate provided a survival benefit in nonhuman primate studies using a homologous, intravenous, pathogenic simian immunodeficiency virus (SIV) challenge [11–14]. Both the VRC HIV-DNA prime and the HIV-rAd5 vaccines were safe, well tolerated, and immunogenic when administered separately to HIV-uninfected adults in the United States [15, 16].

The VRC HIV-DNA and HIV-rAd5 vaccines were evaluated for safety and immunogenicity in 3 studies among lower-risk populations of HIV-uninfected adults in the US, Caribbean, South Africa, and East Africa to establish safety and immunogenicity at sites similar to those that might provide higher-risk volunteers for efficacy testing of the vaccine. The combined data from these studies were considered sufficient to determine whether this candidate vaccine should proceed to proof of concept efficacy testing [10]. Here, we describe one of these studies assessing the safety and immunogenicity of a replication-defective rAd5 administered alone or following priming with a multiclade DNA in East African populations with high titers of preexisting adenovirus 5 (Ad5) neutralizing antibody.

METHODS

Study design. RV 172 was a randomized, double-blinded, placebo-controlled trial performed at 3 clinical research sites: the Makerere University–Walter Reed Project, Kampala, Uganda; the Walter Reed Project, Kericho, Kenya; and the Mbeya Medical Research Programme, Mbeya, Tanzania. The protocol was approved by ethical review boards in each country and the US Department of Defense. All volunteers provided written informed consent. The trial was conducted in 2 parts, as shown in Table 1. Part A, a phase 1 study involving 144 volunteers (groups 1–4), evaluated rAd5 at 2 doses, 10^{10} and 10^{11} particle units (PU)/mL, administered as a single dose alone (groups 1 and 2, respectively) or as a boost after 3 injections of the DNA prime vaccine (groups 3 and 4, respectively). Two-dose regimens of rAd5 were evaluated to provide information about the safety and immunogenicity of this vaccine in African populations, where preexisting neutralizing antibody to the vector is common [17]. Part B, a phase 2 study involving 180 volunteers, further defined safety, tolerability, and immunogenicity for the preferred prime-boost combination for efficacy testing (group 5). In part B, participants were randomized to either vaccine or placebo at a ratio of 1:1. Solicited adverse events commonly associated with vaccination and unsolicited adverse events, including a battery of blood and urine tests, were assessed at clinical encounters with volunteers 2 weeks after each vaccination and 4, 6, 12, and 24 weeks after the final vaccination.

Vaccine product. The VRC vaccines have been described elsewhere [15, 16]. Briefly, the VRC HIV-DNA vaccine (VRC-HIVDNA016–00-VP) and VRC HIV-rAd5 vaccine (VRC-HIVADV014–00-VP) contain largely matched HIV gene inserts, but they are not identical. VRC-HIVDNA016–00-VP (Vaccine Research Center) is composed of 6 closed, circular, DNA plasmids: 3 plasmids expressing HIV-1 Gag, Pol, and Nef proteins from clade B and representing 50% by weight; and 3 plasmids each expressing HIV-1 Env glycoprotein from clades A, B, and C and representing the other 50% by weight (16.67% each). VRC-HIVADV014–00-VP (Vaccine Research Center, Bethesda, Maryland) is composed of 4 replication-defective, recombinant serotype 5 adenoviral vector composed of 4 rAd5 vectors (in a 3:1:1:1 ratio) encoding HIV-1 Gag/Pol polyproteins from clade B and HIV-1 Env glycoproteins from clades A, B, and C. The envelope genes were chosen as representative primary isolates from each of the 3 clades. Mutations and deletions were introduced into HIV genes to preclude expression of functional proteins. Envelope sequences were truncated and the DNA plasmids and rAd5 vectors express gp145 (cytoplasmic

tail deleted) and gp140 (transmembrane domain and cytoplasmic tail deleted) envelope proteins, respectively. In addition, the EnvB construct in the rAd5 had a V1/V2 deletion not present in the DNA product. The DNA vaccine was delivered intramuscularly in a dose of 4 mg using a needle-free injection system (Biojector, Bioject Incorporated). The rAd5 vaccine (1 mL) was delivered intramuscularly by needle injection in a dose of either 10^{10} or 10^{11} PU/mL.

HIV infection diagnosis. Monitoring for HIV-1 infection was performed using a sequential algorithm of the following US Food and Drug Administration approved tests: Genetic Systems rLAV enzyme-linked immunosorbent assay (BioRad Laboratories), Vironostika HIV-1 Microelisa (BioMerieux), Genetic Systems HIV-1 Western Blot Kit (BioRad Laboratories), and Amplicor HIV-1 Monitor, version 1.5, reverse-transcription polymerase chain reaction (Roche Diagnostics).

Adenovirus type 5 neutralizing antibody (Ad5 Nab) assay. Serum samples collected from participants before vaccination were used for determining Ad5 Nab titers. A validated assay was performed at the NIAID Vaccine Immune T cell and Antibody Laboratory (Gaithersburg, Maryland), a contract laboratory supporting NIAID-funded clinical vaccine trials. Serum dilutions, ranging from 1:12 to 1:8748, were incubated with a luciferase reporter rAd5 for 30 min and added to A549 (human lung carcinoma) cells. After incubation for 24 h, luciferase activity was assessed. The 90% inhibition serum titer was determined to be the serum dilution, which can be interpolated to have 10% of the maximum luciferase activity relative to the assay run in the absence of serum.

Cell preparation. Peripheral blood mononuclear cells (PBMCs) were prepared from acid-citrate dextrose anticoagulated tubes by Ficoll-hypaque plus density gradient centrifugation (Pharmacia) using Leucosep tubes (Greiner Bio-one) within 8 h of collection. PBMCs were cryopreserved in Roswell Park Memorial Institute 1640 medium (Sigma) containing 20% heat-inactivated fetal-calf serum and 10% dimethylsulfoxide (DMSO; Sigma) in a Cryo 1°C freezing container (Nalgene). Cells were stored at or below −140°C. Immunogenicity assessments were performed on thawed specimens; average cell viability was >94% immediately after thawing.

IFN-γ ELISpot assay. PBMCs were thawed and rested overnight before testing. The interferon γ (IFN-γ) enzyme-linked immunospot (ELISpot) assay was performed at a central laboratory as described elsewhere [18]. HIV peptides of 15 mer overlapping by 11 amino acids were prepared and divided into 5 peptide pools, representing Env (clades A and B), Pol 1 and Pol 2 (clade B), and Nef (clade B) (Vaccine Research Center). These peptide pools matched the vaccine HIV gene products. An Env C peptide pool was kindly provided by the International AIDS Vaccine Initiative, and HIV clade B-Gag (LAI) was from New England Peptide . All peptides had a purity of >85% as measured by high-performance liquid chromatography and mass spectrometry. All peptides were used at a final concentration of 1 μg/mL. Because all peptides were reconstituted in DMSO, wells containing PBMCs and media only were supplemented with the equivalent concentration of DMSO and served as negative controls. Phytohemagglutinin (PHA; Sigma) was used as a positive control. PBMCs plus peptide antigens or PHA were tested in 3 replicate wells. Negative controls were performed in quadruplicate. Spots were counted with an ImmunoSpot analyzer and software, version 4.0.19 (Cellular Technology Limited). Results are expressed as spot-forming cells (SFCs) per 10^6 PBMCs. A positive IFN-γ response was defined as at least 55 SFC/10^6 PBMCs (uncorrected) and at least 4 times the DMSO-treated wells [19]. The assay was scored as invalid if PHA-treated PBMCs failed to produce a positive response and/or the negative control was ⩾55 SFC/10^6 PBMCs.

Data analysis. Comparisons of reactogenicity and adverse events were made by assigning scores of 0, 1, 2, and 3 to severities of none, mild, moderate, and severe, respectively. The Wilcoxon rank-sum test was used for 2 group comparisons. Otherwise, the Kruskal-Wallis test was used. The Fisher exact test was used for 2×2 tables. All comparisons were based on the maximum severity per volunteer. The proportion of volunteers with positive ELISpot responses was tested using the Fisher exact test. The Cochran-Armitage test for trend was used to compare responses rates across increasing titers of preimmunization Ad5 Nab. The magnitude of ELISpot responses was tested by analysis of variance on $\log_{10}$ response. All tests are 2-tailed, and all analyses were performed using SAS (version 9.2; SAS Institute).

RESULTS

Enrollment and vaccination of 324 volunteers commenced in May 2006 and was completed in October 2006 with 144, 120, and 60 participants at the Ugandan, Kenyan, and Tanzanian sites, respectively. The mean age was 28 years (range, 18–49 years), and 103 (32%) of the participants were female. Three hundred and eleven (96%) participants completed the 12-month study, and 13 (4%) terminated the study before the final visit. Nineteen (6%) participants did not complete the vaccination schedule (8 placebo and 11 vaccine recipients). The most common reason for discontinuation of vaccination was pregnancy ($n = 8$). Two individuals prematurely stopped vaccination due to adverse events (1 vaccine recipient for urticaria and 1 placebo recipient for generalized itching). No deaths

This figure is available in its entirety in the online version of the *Journal of Infectious Diseases.*

Figure 1. Local and systemic reactogenicity to both vaccine and placebo.

Table 2. Frequency (Percentage) of Subjects with Detectable T Cell Responses to Human Immunodeficiency Virus (HIV) Antigens by Treatment Group, as Measured by Interferon-γ Enzyme-Linked Immunospot (ELISpot) Assay in $CD4^+$ or $CD8^+$ T Cells, 6 Weeks after Vaccination

		HIV-1 Peptide Pool					
Treatment	Env A, B, or C	Env A	Env B	Env C	Gag	Pol	Any
All placebos	5/121 (4)	1/120 (1)	2/117 (2)	3/110 (3)	1/119 (1)	1/117 (1)	7/121 (6)
rAd5, 10^{10} PU	15/23 (65)	10/23 (43)	14/22 (64)	3/21 (14)	2/22 (9)	10/22 (45)	17/23 (74)
rAd5, 10^{11} PU	11/23 (48)	7/23 (30)	10/23 (43)	2/21 (10)	1/23 (4)	5/23 (22)	12/23 (52)
rAd5, single dose total	26/46 (57)	17/46 (37)	24/45 (53)	5/42 (12)	3/45 (7)	15/45 (33)	29/46 (63)
D/D/D/rAd5, 10^{10} PU	58/105 (55)	46/105 (44)	48/104 (46)	19/101 (19)	34/105 (32)	14/102 (14)	66/105 (63)
D/D/D/rAd5, 10^{11} PU	12/20 (60)	11/20 (55)	8/18 (44)	4/16 (25)	7/19 (37)	5/18 (28)	12/20 (60)
rAd5, prime boost total	70/125 (56)	57/125 (46)	56/122 (46)	23/117 (20)	41/124 (33)	19/120 (16)	78/125 (62)

NOTE. Data are no. of positive responses/no. of subjects tested (%). D, DNA plasmids (VRC-HIVDNA016–00-VP); PU, particle units; rAd5, recombinant adenovirus 5 vectors (VRC-HIVADV014–00-V).

occurred during the 12 months of the study. A single HIV infection was observed during the study period in a vaccine recipient. All traceable volunteers were offered counseling and HIV testing after public disclosure of the Merck STEP study results, and 264 (81%) attended briefing and voluntary counseling and testing sessions. A total of 4 additional HIV infections have been identified in extended follow-up (1 vaccine recipient and 3 placebo recipients), yielding an overall infection rate of 0.611 per 100 person-years of observation. The infection rate observed by the US Military HIV Research Program in community cohort studies at or near these research sites have ranged from 0.77 to 1.8 per 100 person-years of observation (M. Hoelscher, personal communication; [20]).

Vaccinations were well tolerated (Figure 1). Solicited local and systemic adverse events were commonly reported by both vaccine recipients (88% for both) and placebo recipients (80% for both). The frequency and severity of local and systemic solicited adverse reactions was not different between the high and low dose of rAd5 either alone ($P = .440$ and .367 for local and systemic adverse events, respectively) or in the prime-boost regimen ($P = .458$ and .803 for local and systemic adverse events, respectively). The presence and titer of preexisting antibody to Ad5 did not alter the frequency or severity of solicited local or systemic adverse events among vaccine recipients (data not shown, $P = .767$ and .938, respectively). There were no differences between vaccine and placebo recipients in the frequency or severity of routinely collected clinical laboratory measures (data not shown).

A total of 1446 adverse events were reported among 309 of 324 participants. Infectious diseases were the predominant adverse events, with upper respiratory tract infections being most common, followed by malaria, urinary tract infections, and gas-

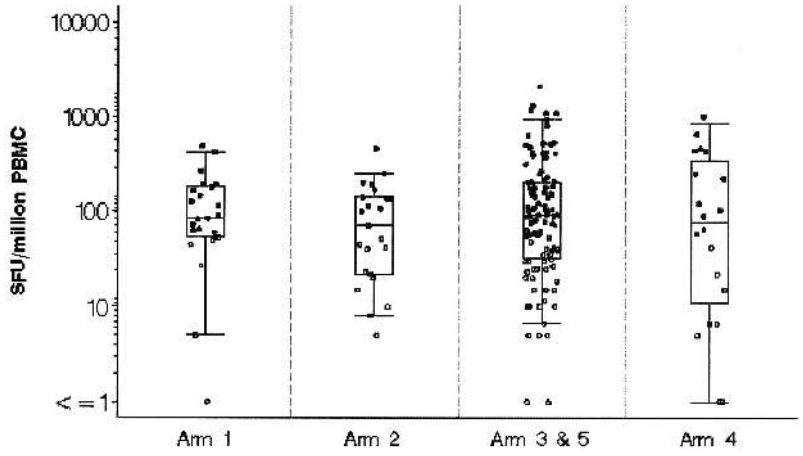

Figure 2. The maximum magnitude of T cell responses measured by interferon-γ enzyme-linked immunospot assay expressed as spot-forming cells (SFCs) per 1 million peripheral blood mononuclear cells (PBMCs) in vaccinees to either Env, Pol, or Gag by vaccination regimen 6 weeks after vaccination. Open circles represent positive responses, and black circles represent negative responses. A positive response was defined as at least 55 spot-forming cells per 10^6 PBMCs in peptide pools and 4 times the background number of SFCs per 10^6 PBMCs (media only). The box plots indicate the median, 25th, and 75th percentile for each group, and the error bars show the 5th and 95th percentiles. Placebo and baseline responses are not shown.

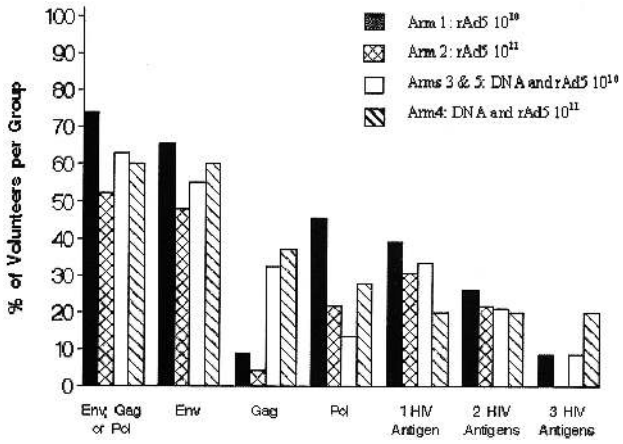

Figure 3. Breadth of T cell responses to human immunodeficiency virus (HIV) antigens. Frequencies of vaccinated subjects with detectable T cell responses to HIV antigens 6 weeks after vaccination. Responses were measured by the interferon γ (IFN-γ) enzyme-linked immunospot (ELISpot) assay. A positive response was defined as at least 55 spot-forming cells (SFCs) per 10^6 peripheral blood mononuclear cells (PBMCs) in peptide pools and 4 times the background number of SFCs per 10^6 PBMCs (media only). The columns represent the percentage of volunteers with a positive IFN-γ ELISpot response for each study group, represented by different colored bars for Env, Gag, or Pol peptides alone; Env alone; either Gag or Pol peptide pools; and for response rates to single, any 2, or any 3 HIV antigens. rAd5, recombinant adenovirus 5.

troenteritis. Blood disorders were the second most common diagnoses, led by neutropenia and thrombocytopenia. Adverse event rates were similar among placebo and vaccine participants. In total, 62 adverse events were graded as severe or life-threatening, and malaria and neutropenia were the most common diagnoses. The proportion of severe or life-threatening adverse events among vaccine and placebo participants were similar (4.9% vs 3.4%; $P = .159$). Four of these adverse events, all of which occurred in vaccine recipients, were considered possibly or probably related to the vaccine. These were 2 cases of generalized urticaria, an episode of neutropenia, and an episode of malaise. There was no relationship between the frequency or severity of adverse events and the titer of preexisting adenovirus antibody for either placebo or vaccine recipients (data not shown).

Immunogenicity assessment results. Responses to HIV antigens were measured using IFN-γ ELISpot assay prior to and 6 weeks after either a single rAd5 injection or the DNA/rAd5 regimen. Results from groups 3 and 5 were combined (arm 3/5), because those groups evaluated the same regimen. Fifteen study participants who did not complete vaccination as assigned per protocol were excluded from analysis, and in some cases, insufficient cells for analysis resulted in additional missing data points.

IFN-γ ELISpot response frequencies after vaccination for each peptide pool and for any positive response are shown for placebo and vaccine recipients by study group in Table 2. False-positive rates were low in the placebo group (7 [6%] of 121), and baseline samples were low for all peptide pools evaluated together (9 [3%] of 275; data not shown). The response rates to any peptide pool for the low and high dose rAd5 alone were 74% and 52%, respectively ($P = .221$). Similarly, response rates for DNA prime and low- or high-dose rAd5 boost were 63% and 60%, respectively ($P = .806$). Positive IFN-γ ELISpot responses to any Env, Gag, or Pol peptide pool at 6 weeks after rAd5 alone (groups 1 and 2) and DNA/rAd5 (groups 3, 4, and 5) were 29 (63%) of 46 and 78 (62%) of 125, respectively and were not significantly different. Response rates for each Env peptide pool were not different between the Ad5 alone and prime-boost groups. The Env pools matching the vaccine amino acid sequence elicited better response rates than the subtype C Env peptide pool regardless of study group. The frequency of Gag responses in the rAd5 alone group was sig-

Table 3. Effect of Pre-existing Adenovirus 5 Neutralizing Antibody Titer on Human Immunodeficiency Virus Type 1 (HIV-1) Specific Interferon-γ Enzyme-Linked Iimmunospot Reactivity

HIV-1 peptide pool	Adenovirus 5 Nab titer	Immunization regimen responders	
		rAd5 only	DNA prime/rAd5 boost
ENV A	<12	2 (50)	5 (71)
	12 to <1000	9 (50)	17 (44)
	1000–5000	3 (25)	21 (51)
	>5000	3 (25)	14 (37)
ENV B	<12	3 (75)	4 (57)
	12 to <1000	13 (76)	17 (44)
	1000–5000	6 (50)	19 (49)
	>5000	2 (17)	16 (43)
ENV C	<12	1 (25)	2 (33)
	12 to <1000	2 (13)	4 (11)
	1000–5000	0 (0)	10 (27)
	>5000	2 (17)	7 (18)
GAG	<12	0 (0)	4 (57)
	12 to <1000	2 (12)	16 (41)
	1000–5000	1 (8)	13 (33)
	>5000	0 (0)	8 (21)
POL	<12	3 (75)	4 (57)
	12 to <1000	7 (41)	5 (13)
	1000–5000	2 (17)	6 (16)
	>5000	3 (25)	4 (11)
Any	<12	3 (75)	6 (86)
	12 to <1000	14 (78)	24 (62)
	1000–5000	7 (58)	27 (66)
	>5000	5 (42)	21 (55)

NOTE. Data are no. (%) of participants, unless otherwise indicated. rAd5, recombinant adenovirus 5.

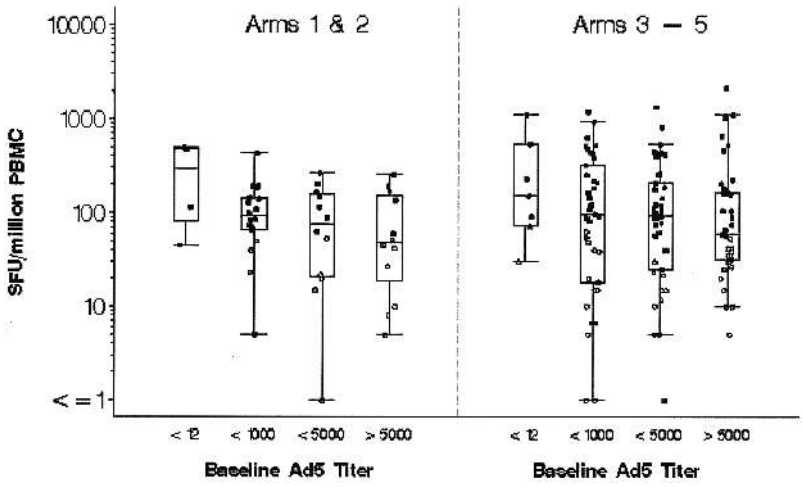

Figure 4. The maximum magnitude of interferon γ enzyme-linked immunospot responses stratified by baseline adenovirus type 5 neutralizing antibody titer of <12, 12–999, 1000–5000, and >5000. Results are expressed as spot-forming cells (SFCs) per 1 million peripheral blood mononuclear cells (PBMCs), for groups 1 and 2 combined and groups 3–5 combined. Open circles represent positive responses, and black circles represent negative responses. A positive response was defined as at least 55 SFCs per 10^6 PBMCs in peptide pools and 4 times the background number of spot-forming cells per 10^6 PBMCs (media only). The box plots indicate the median, 25th, and 75th percentile for each group, and the error bars show the 5th and 95th percentiles. Placebo and baseline responses are not shown. rAd5, recombinant adenovirus 5.

nificantly less than that in the DNA/rAd5 groups (3 of 45 vs 41 of 124; $P < .001$). Pol responses were more common in the rAd5 alone groups than in the DNA/rAd5 groups, (15 of 45 vs 19 of 120; $P = .018$).

There was no difference in the magnitude of response measured by IFN-γ ELISpot assay among the 4 regimens examined in the study ($P = .254$) (Figure 2). The median (range) responses were 127 (60–493), 141 (70–463), 163 (55–2103), and 236 (58–1007) SFC/10^6 PBMCs in arms 1, 2, 3/5, and 4, respectively. There was no difference in terms of response frequency or magnitude between low- and high-dose rAd5 alone (arm 1 vs arm 2) or in a prime/boost format between arm 3/5 and arm 4. Analyzing these data with stratification for titers of preexisting adenovirus antibody showed no significant differences, but these results must be interpreted with caution, given the small numbers of such a comparison (data not shown). The proportions of volunteers with responses to combinations of Env, Gag, and/or Pol peptides are shown in Figure 3. The frequency of positive responses was predominantly to Env, followed by Pol or Gag, regardless of immunization regimen. Among the 4 regimen groups, roughly equal proportions recognized 2 antigens (20%–26%). Group 4, with the high-dose rAd5 boost, had the highest frequency of responders to all 3 antigens tested (20%; $P = .137$).

Ad5 Nab was present at baseline in 175 (94%) of 186 vaccine recipients, with a median titer of 2385. After substituting a value of 8750 for titers reported as >8748, the corresponding median and geometric mean were 3521 and 1407, respectively. Positive IFN-γ ELISpot response rates to HIV Env, Pol, or Gag peptide pools among vaccine recipients only (stratified by Ad5 Nab titer) are shown in Table 3. The frequency of response to any HIV antigen after rAd5 immunization alone was 3 (75%) of 4 for participants with negative Ad5 Nab titers, 14 (78%) of 18 for participants with Ad5 Nab titers of <1000, 7 (58%) of 12 for those with titers 1000–5000, and 5 (42%) of 12 for those with titers >5000 ($P = .048$). Preexisting immunity to Ad5 had no effect ($P = .252$) on HIV-specific response rates in prime and boost recipients, where 6 (86%) of 7 with negative Ad5 Nab titers had a positive IFN-γ ELISpot response, compared with 24 (62%) of 39 with Ad5 Nab <1000, 27 (66%) of 41 with titers 1000–5000, and 21 (55%) of 38 with titers >5000. The magnitude of IFN-γ ELISpot responses for groups 1 and 2 combined and groups 3, 4, and 5 combined and stratified by baseline Ad5 Nab titer is shown in Figure 4. A modest reduction in magnitude with increasing Ad5 Nab titer was apparent, although it was not statistically significant for both single rAd5 vaccination ($P = .184$) and the prime boost regimens ($P = .595$).

DISCUSSION

The field of HIV vaccines has achieved only incremental progress, which has been in the form of negative studies defining conceptual approaches that do not lower acquisition risk or reduce viral load. The benefit of a vaccine that would modestly reduce viral load, even if it confers no protection from acquisition, could be significant. In addition to extending the interval before initiation of antiviral therapy, decreasing host viral bur-

den is likely to reduce secondary HIV transmission [21, 22]. The current study examines the clinical and immunological characteristics of a novel candidate DNA plasmid prime/rAd5 vector boost vaccine combination that is distinct from the vaccines that have failed in test-of-concept efficacy trials [4–6].

The VaxGen monomeric gp120 vaccine was able to induce neutralizing antibody to T cell line adapted variants of HIV but was unable to generate antibody responses to the most commonly transmitted variants of HIV [5, 6]. The Merck vaccine was designed to elicit HIV-specific T cell immunity; it produced HIV-specific responses that were predominantly $CD8^+$ T cell responses, with only 31% of recipients showing both $CD8^+$ and $CD4^+$ T cell responses [23]. In nonhuman primates, the Merck vaccine produced marginal reduction of viral load after SIV challenge [14]. In contrast, the VRC candidate reported here is a heterologous vector strategy that employs a DNA prime and rAd5 boost and has shown both a reduced level of post-challenge viremia and a survival benefit in nonhuman primate SIV challenge studies. Interestingly, this survival benefit was correlated to induction of total HIV-specific T cell responses, $CD8^+$ T cell responses, $CD4^+$ T cell HIV-specific IFN-γ ELISpot responses, and preservation of central memory $CD4^+$ T cells [11, 13]. Similar to observations in the nonhuman primate challenge study, intracellular cytokine staining of a subset of volunteers in this study identifies a balanced $CD4^+$ and $CD8^+$ T cell response to rAd5 after DNA priming [12, 24].

The data presented here show that the VRC rAd5 and DNA/rAd5 HIV vaccines are safe and well tolerated in a population within resource-constrained settings experiencing a considerably greater burden of endemic illness. Vaccination with rAd5 alone or in a prime-boost combination with DNA was highly immunogenic despite very high prevalence and titer of antibody to Ad5 at study entry. A similar study in the United States, which used an rAd5 immunogen dose and regimen identical to the one described here for groups 1 and 2, demonstrated IFN-γ ELISpot response frequencies to HIV antigens that was nearly identical to the responses in our study, supporting the use of this immunogen in a global setting [15]. A diminution in the magnitude of the ELISpot responses with increasing Ad5 Nab has been previously reported for rAd5 vaccines [15, 23]. Although the overall frequency of responders to all HIV antigens was similar between the rAd5-only and prime-boost recipients, there was a reduction in response frequency in the rAd5-only groups with increasing baseline Ad5 titer. The prime-boost strategy induced a higher frequency of responders than did the rAd5 strategy in subjects with rAd5 Nab titers $\geqslant$1000. Surprisingly, there was no evidence of benefit associated with the higher 10^{11} dose of Ad5 vector, compared with the lower 10^{10} dose, in either the rAd5-only or the prime-boost combination group. In terms of overall frequency and magnitude of cellular immune responses as measured by IFN-γ ELISpot assay, the rAd5-only strategy performed as well as the prime-boost regimen, with the exception of Gag responses, where the frequency of responders who received the prime-boost regimen was almost 5 times that of responders who received the rAd5-only vaccination.

It has been argued that a more rigorous animal model would use a challenge that is genetically divergent from the strain used to design the test vaccine. However, it is not known whether the nonhuman primate model will be predictive of protection in humans and, if so, under what experimental conditions. It remains necessary to examine the efficacy of products that are safe and distinct from previous vaccine candidates as a complementary avenue of discovery to validate preclinical testing paradigms. In view of the Step study results, the VRC DNA prime and rAd5 boost vaccine is expected to enter efficacy testing in the United States only among circumcised male subjects without evidence of preexisting immunity to Ad5. Although the population under evaluation in this study is not part of the initial test-of-concept efficacy trial, because of the low rates of circumcision and high prevalence of Ad5 antibody, it will be an important component of other studies should evidence of efficacy be demonstrated.

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