MAJOR ARTICLE

Safety and Immunogenicity of DNA Vaccines Encoding Ebolavirus and Marburgvirus Wild-Type Glycoproteins in a Phase I Clinical Trial

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(See the perspectives article by Levine et al on pages 504–7.)

Background. *Ebolavirus* and *Marburgvirus* cause severe hemorrhagic fever with high mortality and are potential bioterrorism agents. There are no available vaccines or therapeutic agents. Previous clinical trials evaluated transmembrane-deleted and point-mutation *Ebolavirus* glycoproteins (GPs) in candidate vaccines. Constructs evaluated in this trial encode wild-type (WT) GP from *Ebolavirus Zaire* and *Sudan* species and the *Marburgvirus* Angola strain expressed in a DNA vaccine.

Methods. The VRC 206 study evaluated the safety and immunogenicity of these DNA vaccines (4 mg administered intramuscularly by Biojector) at weeks 0, 4, and 8, with a homologous boost at or after week 32. Safety evaluations included solicited reactogenicity and coagulation parameters. Primary immune assessment was done by means of GP-specific enzyme-linked immunosorbent assay.

Results. The vaccines were well tolerated, with no serious adverse events; 80% of subjects had positive enzyme-linked immunosorbent assay results (≥30) at week 12. The fourth DNA vaccination boosted the immune responses.

Conclusions. The investigational *Ebolavirus* and *Marburgvirus* WT GP DNA vaccines were safe, well tolerated, and immunogenic in this phase I study. These results will further inform filovirus vaccine research toward a goal of inducing protective immunity by using WT GP antigens in candidate vaccine regimens.

Clinical Trials Registration. NCT00605514.

Keywords. filovirus; *ebolavirus*; *marburgvirus*; ebola; marburg; DNA; vaccine.

Ebolavirus and *Marburgvirus* belong to the family Filoviridae and are known to cause outbreaks of viral hemorrhagic fever, a severe and often fatal disease. Filoviruses are negative-strand RNA viruses. A single glycoprotein (GP) facilitates viral entry likely through receptor mediated endocytosis into monocytes and macrophages, endothelial cells, and hepatocytes [1–3]. The wild-type (WT) GP antigen is the primary antigen targeted by candidate vaccines.

There are 5 species of *Ebolavirus*: *Zaire* (EBOV), *Sudan* (SUDV), *Reston, Taï Forest,* and *Bundibugyo*. EBOV and SUDV have been responsible for several human outbreaks, with case fatality rates of 41%–90% [4]. They are therefore targeted species for vaccine development. *Marburgvirus* has a single species, *Marburg marburgvirus* with 2 viruses that include Marburg virus (MARV) and Ravn virus, and several strains, including Angola [5], that are currently targeted for vaccine development.

Outbreaks of *Ebolavirus* and *Marburgvirus* have occurred in Africa and have intermittently reemerged, with varying case fatality rates. A 2014 outbreak of

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Ebolavirus disease (species *Zaire ebolavirus*) in West Africa, including Guinea, Sierra Leone, Liberia, and Nigeria, has been the worst outbreak to date and the first to be localized primarily in urban areas [6]. A case fatality rate ranging from 60% to 87% was reported in the first few months of the outbreak. This outbreak affected community members as well as healthcare workers and seems to have spread person to person through regional and international travel. *Marburgvirus* disease has primarily occurred in travelers [4, 5] and, has case fatality rates of 23%–90% [7], the potential to spread internationally with increasing global travel, and the potential threat to be used as a biological weapon.

Several candidate vaccine platforms have been investigated in animal models, including vectors such as vesicular stomatitis virus, DNA plasmids, viruslike particles, and recombinant adenovirus (rAd), alone or as part of a prime-boost strategy [2, 5, 8–11]. Research and development toward a vaccine that would provide protective immunity against these infections has been an iterative process requiring the clinical evaluation of interim candidate GP vaccine antigens, in part because of theoretical safety concerns.

In 2006, we reported the first clinical trial evaluating a multigene DNA vaccine encoding transmembrane-deleted GP from EBOV and SUDV and nucleoprotein from EBOV [12]. The vaccine was well tolerated, with no significant adverse events or coagulation abnormalities. The vaccine elicited GP-specific antibody and T-cell responses that were not cross-reactive, but after further preclinical evaluation of GP antigens, we found that a transmembrane-deleted GP did not provide optimal protection and that the nucleoprotein antigen was not required for protection.

The subsequent clinical trial evaluated an rAd5 vector vaccine expressing an EBOV GP with a single amino acid point mutation (point mutation GP). The product was found to be safe and well tolerated but elicited modest immunogenicity, possibly in part because of suppression by preexisting immunity to the Ad5 vector [13]. Nonhuman primate studies have further shown that transmembrane-deleted and point mutation GP antigens are partially protective but WT GP constructs are safe and provide the highest level of protection [14]. Therefore, the WT GP antigen is the current focus of Vaccine Research Center (VRC) research and development for *Ebolavirus* and *Marburgvirus* vaccines. Here we report the results of a phase I clinical trial evaluating 2 DNA vaccines, one that encodes for MARV Angola GP and the second for EBOV and SUDV WT GP.

MATERIALS AND METHODS

Study Design and Procedures

VRC 206 was a single-site, phase 1, open label study examining the safety, tolerability, and immunogenicity of 2 investigational DNA vaccines, one (MAR) expressing GP from MARV Angola strain (GP [AN]) and the other (EBO) expressing WT GP from EBOV (GP [Z]) and SUDV (GP [S]) in healthy adults aged 18–60 years. The study was conducted at the NIH Clinical Center by the VRC, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, Maryland (clinicaltrials.gov NCT00605514). The study was reviewed and approved by the NIAID Institutional Review Board. The US Department of Health and Human Services human experimental guidelines for conducting clinical research were followed. All subjects gave written informed consent before participation.

A schema of the study is shown in Figure 1. The study groups were not randomized because approval to proceed with the MAR DNA vaccine was received before approval to proceed with the EBO WT DNA vaccine owing to a delay in receiving preclinical data with the EBO WT DNA vaccine. Thus, group 1 was fully enrolled first to receive the MAR DNA vaccine. Later, group 2 enrolled to receive the EBO WT DNA vaccine. No more than 1 subject per day was administered vaccine for the first 3 injections in each group, and safety data through 2 weeks after these injections were reviewed by a protocol safety review team before continuing enrollment of that group.

A 4-mg dose of vaccine was administered as 1 mL by intramuscular injection in the deltoid muscle, using the Biojector 2000 Needle-Free Injection Management System (Bioject). In both groups, the immunization series was a 3-dose priming regimen with an optional single-dose homologous booster. The schedule for the 3-dose priming series was targeted to study days 0, 28, and 56, within permitted windows. Based on results from preclinical immunogenicity data available after the VRC 206 study began, an optional homologous booster dose at week 32 or later was offered to subjects who had completed all 3 injections and remained in clinical follow-up.

Laboratory and clinical evaluations were completed at scheduled study visits. Local and systemic reactogenicity was self-reported by subjects using 5-day diary cards after each vaccination. Clinical assessment and laboratory evaluations for creatinine, alanine aminotransferase, complete blood cell count, prothrombin time, and partial thromboplastin time were completed at scheduled study visits. Adverse events were reported for the entire duration of the study, coded using the *Medical Dictionary for Regulatory Activities* and graded for severity using the Division of AIDS table for grading the severity of adverse events (December 2004) [15]. Subjects were followed up for safety and immunogenicity for 32 weeks or for 12 weeks after receipt of the optional fourth study injection.

Vaccines

Both the EBO plasmid DNA vaccine VRC-EBODNA023-00-VP and the MAR plasmid DNA vaccine VRC-MARDNA025-00-VP were developed by the VRC. The EBO plasmid DNA vaccine was manufactured by the VRC/NIAID Vaccine Pilot Plant, operated by Leidos in Frederick, Maryland, and the MAR plasmid DNA vaccine was produced by Althea Technologies in San Diego, California. Both were manufactured under good manufacturing practice conditions. The MAR vaccine

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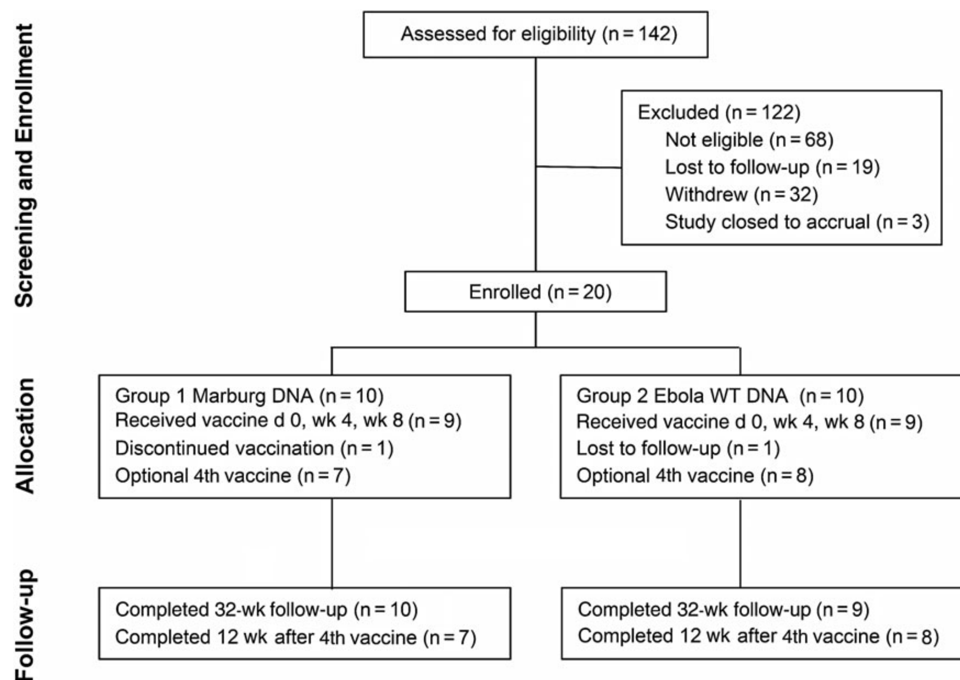

Figure 1. Schematic diagram of study design, vaccination schedule and follow-up showing screening, enrollment, and follow-up. The original protocol design included 3 study vaccinations at day 0 and weeks 4 and 8, with 32 weeks of follow-up. An optional fourth vaccination was added by amendment with 12 weeks of additional follow-up. Abbreviation: WT, wild-type.

consists of a single, closed, circular plasmid DNA macromolecule (plasmid VRC 6712) designed to express WT GP of the MARV Angola strain (GenBank accession No. DQ447653). The EBO vaccine is composed of 2 plasmids combined in equal concentrations (milligrams per milliliter) in which the amino acid sequences for the GP antigens expressed are identical to WT GP of *Ebolavirus*. These plasmids are VRC 6611, which expresses the GP gene from the SUDV strain (GenBank accession No. U28134) and VRC 6614, which expresses the GP gene from the EBOV strain (GenBank accession No. U23187). The WT GP inserts were codon modified to optimize antigen expression in human cells [16]. The plasmids in both vaccines are incapable of replication in human cells.

The VRC constructed plasmids containing complementary DNAs used to subclone the WT GP gene inserts into the CMV/R [12] plasmid DNA expression vector [17]. This CMV/R expression vector has been tested in previous clinical trials of DNA plasmid vaccines for human immunodeficiency virus, West Nile virus, severe acute respiratory syndrome, and avian influenza conducted by the VRC [12, 18–21].

The DNA plasmids were produced in master cell banks using plasmid DNA to transform commercially available strains of *Escherichia coli* and produce large-volume cultures yielding mass quantities of plasmid DNA. Bacterial cell growth was dependent on the cellular expression of kanamycin resistance protein encoded in the plasmid DNA. After the growth of bacterial cells, the bulk plasmid drug substances were filtered and formulated in phosphate-buffered saline as a sterile liquid injectable dosage form for intramuscular injection.

Measurement of Antibody Responses With Enzyme-Linked Immunosorbent Assays

End point titers of antibodies directed against MAR GP (AN) and EBO GP antigens (Z and S) were measured throughout the study. Serial dilutions were analyzed in duplicate for pre- and postvaccine time points. Mean raw optical density values for each postvaccine time point were corrected for the volunteer and dilution-matched samples. End point titers were calculated as the most dilute serum concentration that gave a background-corrected optical density reading of >0.20. A titer of ≥30 was considered a positive result [12, 13]. The primary time points were 4 weeks after the third and the fourth study injections.

Peptides for Evaluation of T-Cell Responses

Peptides 15 amino acids in length, overlapping by 11, and corresponding to the vaccine inserts, MAR GP (AN) and EBO GP antigens (Z and S), were synthesized at >80% purity, as confirmed by high-performance liquid chromatography.

Measurement of T-Cell Responses by Enzyme-Linked Immunospot Assay

Vaccine-induced T-cell responses were detected by enzyme-linked immunospot assay (ELISPOT) with the following modifications of a method described elsewhere [22]. Peripheral blood mononuclear cells (PBMCs) were stimulated overnight

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Table 1. Baseline Demographics

Characteristic	Subjects, No. (%)[a]		
	Marburgvirus Vaccine (n = 10)	*Ebolavirus* WT Vaccine (n = 10)	All Subjects (n = 20)
Sex			
Male	5 (50)	5 (50)	10 (50)
Female	5 (50)	5 (50)	10 (50)
Age, mean (SD) [range], y	34.0 (9.7) [24–52]	36.6 (11) [24–59]	35.3 (10) [24–59]
Race			
White	9 (90)	7 (70)	16 (80)
Black or African American	1 (10)	1 (10)	2 (10)
Asian	0	1 (10)	1 (5)
All other races combined	0	1 (10)	1 (5)
Ethnicity			
Non-Hispanic/Latino	10 (100)	10 (100)	20 (100)
Hispanic/Latino	0	0	0
BMI, mean (SD) [range]	24.6 (2.4) [21.4–28.5]	25.8 (4.7) [20.0–33.4]	25.2 (3.7) [20.0–33.4]
Educational level			
Less than high school	0	0	0
High school/GED	0	1 (10)	1 (5)
College/university	2 (20)	3 (30)	5 (25)
Advanced degree	8 (80)	6 (60)	14 (70)

Abbreviations: BMI, body mass index; GED, General Educational Development; SD, standard deviation; WT, wild type.

[a] Unless otherwise indicated, data represent No. (%) of subjects.

with 2.5 μg/mL peptide at 37°C in triplicate wells at a density of 2×10^5 cells per well. Commercially available ELISPOT plates (Mabtech) were used with color development with biotinylated interferon (IFN) γ detection antibodies, avidin–horseradish peroxidase solution, and 3-amino, 9 ethyl-carbazole substrate solution. The plate was air dried for ≥2 hours before spot quantitation on a CTL ELISPOT image analyzer (Cellular Technology). Results were expressed as mean spot-forming cells per million PBMCs. A positive response occurs if the background subtracted number of spots per 1×10^6 cells is >100 spot-forming cells per million PBMCs and the non–background-corrected mean is ≥4-fold greater than the mean negative stimulation for the sample.

Measurement of T-Cell Responses With Intracellular Cytokine Staining

Vaccine-induced T-cell responses were detected by intracellular cytokine staining with the following modifications of a method described elsewhere [17]. Antibodies were from BD Biosciences unless otherwise stated and included anti-CD28-Cy5PE [cyanine dye Cy5™, phycoerythrin (PE)], anti-CD45RA-Cy7PE [cyanine dye Cy7™, PE], anti-CCR7-Ax680 [Alexa Fluor® 680] (ReaMetrix), anti-IFN-γ-APC [allophycocyanin (APC)], anti-IL-2-PE, anti-TNF-α-FITC [fluorescein isothiocyanate (FITC)], anti-CD4-ECD [PE-Texas Red®-x] (Beckman Coulter), anti-CD3-Cy7APC [cyanine dye Cy7™, APC], anti-CD8-Pacific Blue, and Aqua Blue. Cells were stained with Aqua Blue at room temperature for 20 minutes, followed immediately by staining with the surface markers (CD3, CD28, CD45RA, and CCR7) for another 20 minutes. Cells were washed twice, permeabilized with CytoFix-CytoPerm reagent (BD; 100 μL per well) with 20-minute incubation at 2°C –8°C, and then washed twice with PermWash (BD). Intracellular cytokine staining (CD4, CD8, IFN-γ, interleukin 2, tumor necrosis factor α) was in a total of 100 μL per well at room temperature for 20 minutes, followed by 3 washes with PermWash. The cells were resuspended in 1% paraformaldehyde and stored at 4°C for ≤36 hours before flow cytometric analysis. Multiparameter flow cytometric analysis was performed on a LSR-II flow cytometer (BDIS); 50 000–250 000 events were acquired.

Results were analyzed using FlowJo software Version 9.4.10 (Tree Star Software). The same gating strategy is used for all clinical testing. A positive response occurs if a Fisher exact test for the 2×2 table, consisting of positive and negative cells by peptide and negative control, has a 1-sided P value <.01, and the percentage of positive cells for a peptide minus the percentage of positive cells for the negative control (background subtracted percentage) exceeds 0.05 for each combination of cell population and cytokine, except for CD8 IFN-γ and CD8 tumor necrosis factor α, with a threshold of >0.08.

Neutralizing Antibody Assay

The measurement of neutralizing antibodies elicited after vaccination was assessed as described elsewhere [13]. In brief, EBOV or MARV GP-specific neutralizing antibody was assessed by using

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a single-round infection assay with EBOV or MARV GP-pseudotyped lentiviruses, respectively, containing the luciferase reporter gene. The derivative T-Ag–expressing 293T cells were used as infection targets and incubated in a 96-well plate 1 day before infection with pseudovirus in the presence of a 1:100 dilution of subject serum samples. EBOV or MARV GP-pseudotyped lentiviral virions were produced as described elsewhere [16]. Pre- and postimmune serum samples were tested as indicated in the figure legends. Cells were lysed 72 hours after infection and assayed with the Luciferase Assay System (Promega, E1501/E1531), using a Victor X3 Plate Reader from PerkinElmer to detect luciferase activity.

Statistical Methods

Positive response rates are computed along with the exact 95% confidence intervals (CIs) from binomial distributions. For the magnitude of antibody response, geometric means and 95% CIs based on lognormal distribution are reported. The Wilcoxon signed rank test is used to compare antibody response at 2 time points within a group.

RESULTS

Study Population

A total of 20 subjects were enrolled between 4 June 2008 and 17 June 2009. Table 1 includes demographic data regarding sex, age, race, ethnicity, body mass index, and educational level at enrollment. All but 2 subjects completed the 3-dose vaccination series. One subject in group 1 discontinued vaccinations per protocol requirements after 1 study vaccination owing to a medical need for a licensed vaccine, and 1 subject in group 2 was lost to follow-up after 2 vaccinations.

Vaccine Safety

Both vaccines were well tolerated, and there were no serious adverse events. Coagulation parameters of study subjects were

Table 2. Local Reactogenicity by Vaccine Group and Overall[a]

Local Symptom Intensity	Subjects, No. (%)		
	Marburgvirus Vaccine (n = 10)	*Ebolavirus* WT Vaccine (n = 10)	All Subjects (n = 20)
Pain/tenderness			
None	1 (10)	3 (30)	4 (20)
Mild	8 (80)	7 (70)	15 (75)
Moderate	1 (10)	0	1 (5)
Severe	0	0	0
Swelling			
None	5 (50)	7 (70)	12 (60)
Mild	5 (50)	3 (30)	8 (40)
Moderate	0	0	0
Severe	0	0	0
Redness			
None	6 (60)	6 (60)	12 (60)
Mild	4 (40)	4 (40)	8 (40)
Moderate	0	0	0
Severe	0	0	0
Any local symptom			
None	1 (10)	3 (30)	4 (20)
Mild	8 (80)	7 (70)	15 (75)
Moderate	1 (10)	0	1 (5)
Severe	0	0	0

Abbreviation: WT, wild type.

[a] Local and systemic reactogenicity was self-reported by subjects using 5-day diary cards after each vaccination.

Table 3. Systemic Reactogenicity by Vaccine Group and Overall[a]

Systemic Symptom Intensity	Subjects, No. (%)		
	Marburgvirus Vaccine (n = 10)	*Ebolavirus* WT Vaccine (n = 10)	All Subjects (n = 20)
Malaise			
None	4 (40)	8 (80)	12 (60)
Mild	4 (40)	2 (20)	6 (30)
Moderate	2 (20)	0	2 (10)
Severe	0	0	0
Myalgia			
None	7 (70)	10 (100)	17 (85)
Mild	2 (20)	0	2 (10)
Moderate	1 (10)	0	1 (5)
Severe	0	0	0
Headache			
None	6 (60)	9 (90)	15 (75)
Mild	4 (40)	1 (10)	5 (25)
Moderate	0	0	0
Severe	0	0	0
Chills			
None	9 (90)	9 (90)	18 (90)
Mild	1 (10)	1 (10)	2 (10)
Moderate	0	0	0
Severe	0	0	0
Nausea			
None	8 (80)	10 (100)	18 (90)
Mild	2 (20)	0	2 (10)
Moderate	0	0	0
Severe	0	0	0
Temperature			
None	10 (100)	10 (100)	20 (100)
Mild	0	0	0
Moderate	0	0	0
Severe	0	0	0
Any systemic symptom			
None	3 (30)	7 (70)	10 (50)
Mild	5 (50)	3 (30)	8 (40)
Moderate	2 (20)	0	2 (10)
Severe	0	0	0

Abbreviation: WT, wild type.

[a] Local and systemic reactogenicity was self-reported by subjects using 5-day diary cards after each vaccination.

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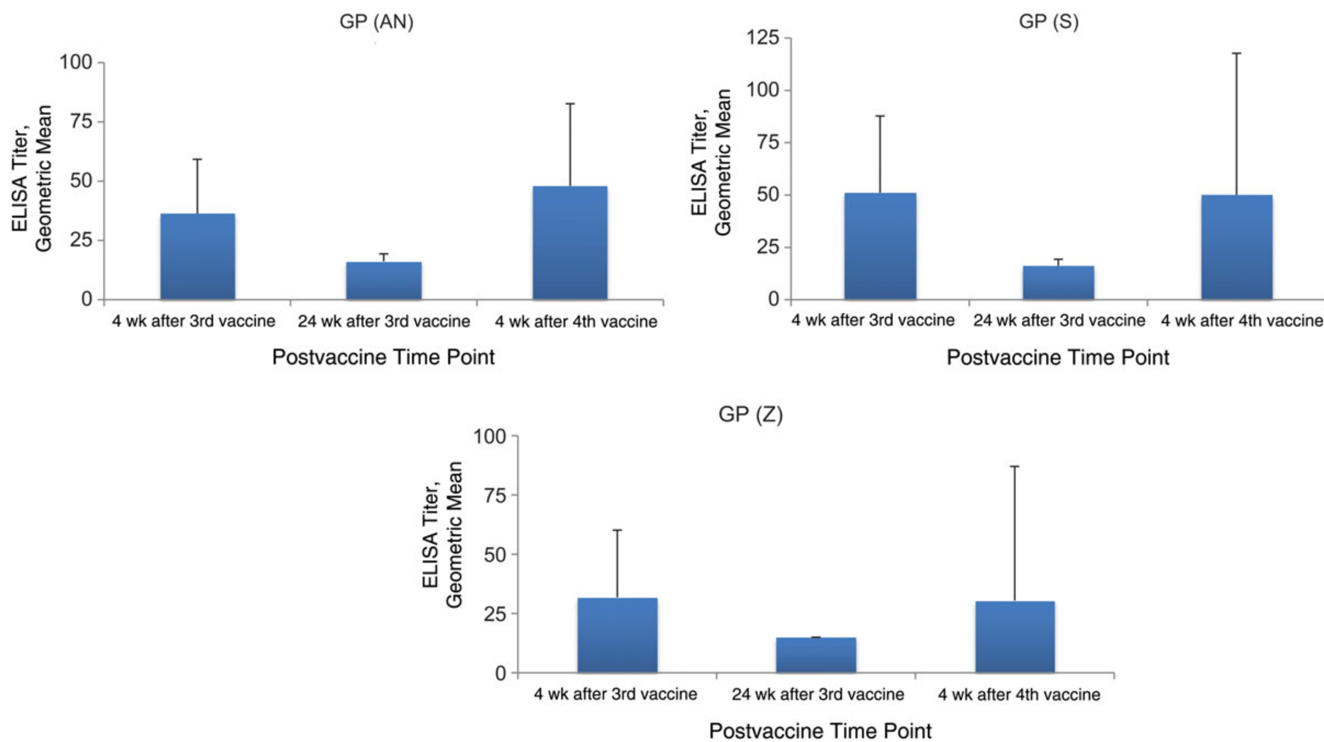

Figure 2. Glycoprotein (GP)-specific mean antibody responses assessed by enzyme-linked immunosorbent assay (ELISA) for GP (AN), GP (S), and GP (Z). Mean titers with upper 95% confidence intervals are shown for 3 time points: 4 and 24 weeks after the third vaccination and 4 weeks after the fourth DNA vaccination. The threshold for positivity in this assay was a reciprocal dilution of 30 and is indicated by a dashed line.

closely monitored as per protocol owing to a theoretical concern over GP-mediated cytopathology [13]. Prothrombin time, partial thromboplastin time, and complete cell blood counts were evaluated at baseline and throughout the study. No laboratory or clinical coagulation abnormalities were detected. Local and systemic solicited reactogenicity (Tables 2 and 3) was generally mild in both groups and similar to that seen in previous studies of DNA vaccines with other constructs [12, 18, 21, 23]. One subject reported a moderate local reaction, and 2 reported ≥1 moderate systemic reaction. Two adverse events, mild transient lightheadedness and mild pyuria, were assessed as possibly related to vaccination. One adverse event, a mild superficial lesion at the injection site that resolved without treatment, was assessed as definitely related to the study vaccines based on temporal relationship and biological plausibility.

Vaccine-Specific Antibody Responses

EBO and MAR specific antibody responses were detected by enzyme-linked immunosorbent assay. Four weeks after the third vaccination, 80% of subjects (8 of 10) in group 1 tested positive for GP (AN) antibody. In group 2, 89% (8/9) tested positive for GP (S) and 56% (5/9) tested positive for GP (Z). The frequency of responses decreased over time, and 24 weeks after the third vaccination 11% of subjects (1 of 9) were positive for GP (AN) and GP (S), and none were positive for GP (Z).

Seven subjects in group 1 and 8 in group 2 received the optional fourth vaccine (homologous boost) between study weeks 32 and 49. Four weeks after the fourth vaccination, 100% of subjects (7 of 7) were positive for GP (AN), 75% (6/8) for GP (S), and 63% (5/8) for GP (Z). Between 8 and 12 weeks after the boost, 57% (4 of 7) in group 1 had sustained positive responses for GP (AN), and 50% (4 of 8) in group 2 had sustained positive responses for GP (S) and GP (Z). Cumulative GP-specific response rates in the study were 90% (9 of 10) for GP (AN), 89% (8 of 9) for GP (S), and 67% (6 of 9) for GP (Z).

The magnitude of enzyme-linked immunosorbent assay titers peaked 4 weeks after the third vaccination and then decreased during the next 24 weeks. The geometric mean titers to GP (AN), GP (S), and GP (Z) 4 weeks after the third vaccination were 36.3 (95% CI, 22.3–59.3), 51.1 (95% CI, 29.8–87.8), and 31.8 (95% CI, 16.8–60.2), respectively, and then decreased close to baseline levels. Four weeks after the fourth vaccination, geometric mean titers to GP (AN), GP (S), and GP (Z) increased to 48.0 (95% CI, 27.9–82.7), 50.1 (95% CI, 21.3–117.7), and 30.4 (95% CI, 16.4–56.1), respectively (Figure 2).

Antibody responses were boosted by the fourth DNA vaccination. Administration of a fourth homologous DNA vaccine at a boost interval of ≥32 weeks demonstrated boosting of waning antibody responses to near peak levels.

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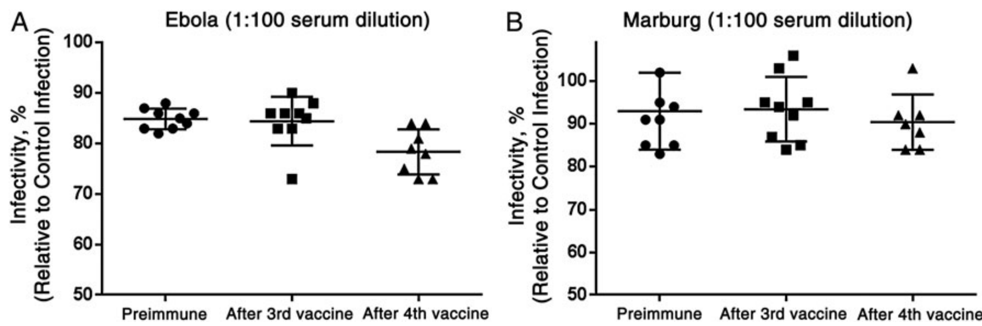

Figure 3. *Ebolavirus* and *Marburgvirus* neutralization as the percentage of infection in the presence of subject serum samples relative to control infection. Neutralization is represented as the percentage of infection in the presence of subject serum samples relative to control infection in the absence of serum samples. Results are shown for 9 of 10 EBO vaccinees (*A*) and 9 of 10 MAR vaccinees (*B*) at 3 time points: at baseline (preimmune) and after the third and fourth DNA vaccinations.

GP (AN)– and GP (Z)–specific neutralizing antibody activity was assessed in a reporter virus assay. Results are shown in Figure 3 for each group at baseline (preimmune) and after the third and fourth DNA vaccinations. No significant MAR neutralizing activity was observed. Low-level EBOV neutralizing activity (mean, 7%) was observed after the fourth vaccination, which was significantly improved compared with preimmune serum samples or after the third vaccination.

Vaccine-Specific T-Cell Responses

MAR and EBO GP-specific CD4$^+$ T-cell responses were seen in all antigen groups by intracellular cytokine staining at 4 weeks after the third DNA vaccination. CD4$^+$ T-cell responses were detected for GP (AN) in 30% of subjects (3 of 10), GP (S) in 25% (2 of 8), and GP (Z) in 13% (1 of 8). By week 24, 22% (2 of 9) still had a CD4$^+$ T-cell response for GP (AN) and GP (S), and 11% (1/9) for GP (Z). Four weeks after the fourth DNA vaccination, the frequency was similar at 14% (1 of 7) for GP (AN), 38% (3 of 8) for GP (S), and 13% (1 of 8) for GP (Z) (Figure 4).

CD8$^+$ T-cell responses were detected less frequently than CD4$^+$ T-cell responses. Four weeks after the third vaccination, CD8$^+$ T-cell responses were detected in 13% of subjects (1 of 8) for GP (S) but not detected for GP (AN) or GP (Z). Four weeks after the fourth vaccination, the frequency of measurable CD8$^+$ T-cell responses was boosted to 25% of subjects (2 of 8) for GP (S) and 13% (1 of 8) for GP (Z), although none were seen for GP (AN).

GP-specific T-cell responses were also assessed by ELISPOT at baseline and at scheduled intervals throughout the study. Four weeks after the third DNA vaccination, GP-specific

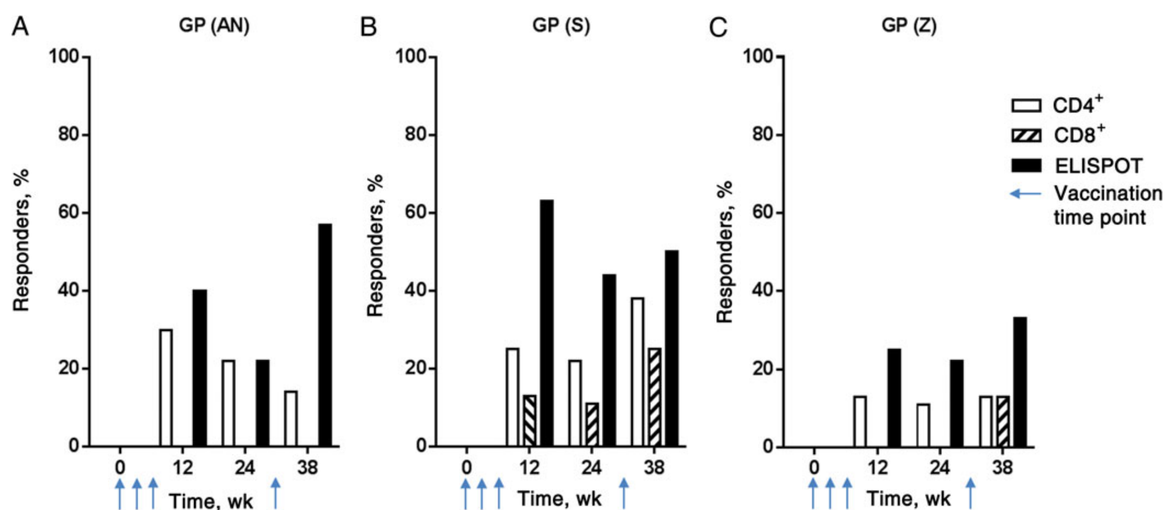

Figure 4. Frequency of CD4$^+$ and CD8$^+$ T-cell responses by intracellular cytokine staining and enzyme-linked immunospot assay (ELISPOT) analysis. The percentage of responders is shown for each vaccine antigen at 4 time points for CD4$^+$ and CD8$^+$ intracellular cytokine staining and ELISPOT.

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T-cell responses were present at a frequency of 40% (4 of 10) for GP (AN), 63% (5 of 8) for GP (S), and 25% (2 of 8) for GP (Z). At 24 weeks, 22% of subjects (2 of 9) were positive for GP (AN), 44% (4 of 9) were positive for GP (S), and 22% (2 of 9) were positive for GP (Z). Four weeks after the fourth vaccination, the frequency of T-cell responses increased to 57% (4 of 7) for GP (AN), 50% (4 of 8) for GP (S), and 33% (3 of 8) for GP (Z), indicating a slight boost in T-cell responses with the fourth DNA vaccination.

DISCUSSION

There are currently no available effective vaccines or therapies against filoviruses. An effective vaccine would be a key preventive health measure for limiting the spread of infection, protecting healthcare workers and military troops, and containing natural outbreaks.

This is the first clinical trial report of an *Ebolavirus* WT GP construct and the first report of a *Marburgvirus* vaccine clinical trial. We have shown elsewhere that earlier-generation gene-based constructs were safe and immunogenic, including a DNA vaccine with an EBO transmembrane-deleted GP and an rAd5 vector encoding EBO GP containing a point mutation. Nonhuman primate studies have clearly demonstrated that full-length WT GP constructs are safe and protective in cynomolgous macaques [14], and WT GP is considered the antigen most likely to induce protective antibody and T-cell responses. In this phase I study (VRC 206), both the EBO and MAR WT GP vaccines were well tolerated. The WT GP constructs evaluated in the current study were immunogenic and induced both humoral and T-cell responses to all 3 GP immunogen inserts.

We also saw that administration of a fourth dose of DNA as a homologous boost improved the otherwise waning antibody titers and T-cell responses. It is known that DNA provides a priming response for numerous antigens, typically with a protein or more potent vector boost, and that a prolonged boost interval improves the overall effect of DNA priming [24], but this report is unique in that homologous DNA vaccine induced a demonstrable boost of preexisting memory B cells.

In the current trial, antibody responses dominated the immune response, and $CD4^+$ T-cell responses were more frequent than $CD8^+$ responses. The induction of T-cell responses by the vaccine is significant because recent nonhuman primate studies suggest that $CD8^+$ T-cell responses play an important role in protection induced by an EBO GP construct vaccine and are known to be important for efficient viral clearance [25, 26].

This study (VRC 206) demonstrated that WT GP DNA vaccines for *Ebolavirus* and *Marburgvirus* were safe and immunogenic in humans. The results from this study paved the way for further evaluation of these 2 candidate vaccines in the first clinical trial of candidate *Ebolavirus* and *Marburgvirus* vaccines in Africa. A phase Ib clinical trial evaluating these vaccines opened to accrual in Kampala, Uganda, after interim safety analysis of the VRC 206 study. The successful evaluation of DNA vaccines targeting *Ebolavirus* and *Marburgvirus* reported here provides the opportunity to further explore WT filovirus GP antigen delivery in other vaccine platforms with greater immunogenicity and potential for protective immunity. These results will guide further filovirus vaccine research and development and also provide important generalizable data regarding DNA-based priming and boosting in humans.

Notes

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References

1. Sanchez A, Kiley MP, Klenk HD, Feldmann H. Sequence analysis of the Marburg virus nucleoprotein gene: comparison to Ebola virus and other non-segmented negative-strand RNA viruses. J Gen Virol **1992**; 73(pt 2):347–57.
2. Hart MK. Vaccine research efforts for filoviruses. Int J Parasitol **2003**; 33:583–95.
3. Dessen A, Volchkov V, Dolnik O, Klenk HD, Weissenhorn W. Crystal structure of the matrix protein VP40 from Ebola virus. EMBO J **2000**; 19:4228–36.
4. Centers for Disease Control and Prevention. Ebola (Ebola Virus Disease): Outbreaks. Known cases and outbreaks of Ebola hemorrhagic fever, in chronological order. Available at: http://www.cdc.gov/vhf/ebola/outbreaks/index.html. Accessed 12 August 2014.
5. Sarwar UN, Sitar S, Ledgerwood JE. Filovirus emergence and vaccine development: a perspective for health care practitioners in travel medicine. Travel Med Infect Dis **2011**; 9:126–34.
6. Centers for Disease Control and Prevention. 2014 Ebola outbreak in West Africa. http://www.cdc.gov/vhf/ebola/outbreaks/guinea/. Accessed 12 August 2014.
7. Centers for Disease Control and Prevention. Marburg hemorrhagic fever (Marburg HF). http://www.cdc.gov/vhf/marburg/index.html. Accessed 12 August 2014.
8. Jones SM, Feldmann H, Stroher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. Nat Med **2005**; 11:786–90.
9. Daddario-DiCaprio KM, Geisbert TW, Geisbert JB, et al. Cross-protection against Marburg virus strains by using a live, attenuated recombinant vaccine. J Virol **2006**; 80:9659–66.

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10. Daddario-DiCaprio KM, Geisbert TW, Stroher U, et al. Postexposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment. Lancet **2006**; 367:1399–404.
11. Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. Nature **2000**; 408:605–9.
12. Martin JE, Sullivan NJ, Enama ME, et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol **2006**; 13:1267–77.
13. Ledgerwood JE, Costner P, Desai N, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine **2010**; 29:304–13.
14. Sullivan NJ, Geisbert TW, Geisbert JB, et al. Immune protection of non-human primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. PLoS Med **2006**; 3:e177.
15. Division of AIDS Table for Grading the Severity of Adverse Events (December 2004). Available at: http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/daidsaegradingtable.pdf.
16. Yang ZY, Huang Y, Ganesh L, et al. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol **2004**; 78:5642–50.
17. Barouch DH, Yang ZY, Kong WP, et al. A human T-cell leukemia virus type 1 regulatory element enhances the immunogenicity of human immunodeficiency virus type 1 DNA vaccines in mice and nonhuman primates. J Virol **2005**; 79:8828–34.
18. Catanzaro AT, Roederer M, Koup RA, et al. Phase I clinical evaluation of a six-plasmid multiclade HIV-1 DNA candidate vaccine. Vaccine **2007**; 25:4085–92.
19. Ledgerwood JE, Hu Z, Gordon IJ, et al. Influenza virus h5 DNA vaccination is immunogenic by intramuscular and intradermal routes in humans. Clin Vaccine Immunol **2012**; 19:1792–7.
20. Ledgerwood JE, Pierson TC, Hubka SA, et al. A West Nile virus DNA vaccine utilizing a modified promoter induces neutralizing antibody in younger and older healthy adults in a phase I clinical trial. J Infect Dis **2011**; 203:1396–404.
21. Martin JE, Louder MK, Holman LA, et al. A SARS DNA vaccine induces neutralizing antibody and cellular immune responses in healthy adults in a phase I clinical trial. Vaccine **2008**; 26:6338–43.
22. Graham BS, Koup RA, Roederer M, et al. Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 DNA candidate vaccine. J Infect Dis **2006**; 194:1650–60.
23. Martin JE, Pierson TC, Hubka S, et al. A West Nile virus DNA vaccine induces neutralizing antibody in healthy adults during a phase 1 clinical trial. J Infect Dis **2007**; 196:1732–40.
24. Ledgerwood JE, Wei CJ, Hu Z, et al. DNA priming and influenza vaccine immunogenicity: two phase 1 open label randomised clinical trials. Lancet Infect Dis **2011**; 11:916–24.
25. Fausther-Bovendo H, Mulangu S, Sullivan NJ. Ebolavirus vaccines for humans and apes. Curr Opin Virol 2012; 2:324–9.
26. Sullivan NJ, Hensley L, Asiedu C, et al. $CD8^+$ cellular immunity mediates rAd5 vaccine protection against Ebola virus infection of nonhuman primates. Nat Med 2011; 17:1128–31.

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