ORIGINAL ARTICLE

Chimpanzee Adenovirus Vector Ebola Vaccine

Julie E. Ledgerwood, D.O., Adam D. DeZure, M.D., Daphne A. Stanley, M.S., Emily E. Coates, Ph.D., Laura Novik, M.A., Mary E. Enama, M.A., Nina M. Berkowitz, M.P.H., Zonghui Hu, Ph.D., Gyan Joshi, M.S., Aurélie Ploquin, Ph.D., Sandra Sitar, M.S., Ingelise J. Gordon, R.N., Sarah A. Plummer, C.R.N.P., LaSonji A. Holman, F.N.P., Cynthia S. Hendel, C.R.N.P., Galina Yamshchikov, M.S., Francois Roman, M.D., Alfredo Nicosia, Ph.D., Stefano Colloca, Ph.D., Riccardo Cortese, M.D., Robert T. Bailer, Ph.D., Richard M. Schwartz, Ph.D., Mario Roederer, Ph.D., John R. Mascola, M.D., Richard A. Koup, M.D., Nancy J. Sullivan, Ph.D., and Barney S. Graham, M.D., for the VRC 207 Study Team*

From the Vaccine Research Center (J.E.L., A.D.D., D.A.S., E.E.C., L.N., M.E.E., N.M.B., A.P., S.S., I.J.G., S.A.P., L.A.H., C.S.H., G.Y., R.T.B., R.M.S., M.R., J.R.M., R.A.K., N.J.S., B.S.G.) and the Biostatistics Research Branch, Division of Clinical Research (Z.H., G.J.), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD; GlaxoSmithKline Vaccines, Rixensart, Belgium (F.R.); ReiThera, Rome (A.N., S.C.), and CEINGE and the Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples (A.N.) — both in Italy; and Keires, Basel, Switzerland (R.C.). Address reprint requests to Dr. Ledgerwood at ledgerwood@mail.nih.gov.

*A complete list of members of the VRC 207 Study Team is provided in the Supplementary Appendix, available at NEJM.org.

Drs. Sullivan and Graham contributed equally to this article.

A preliminary version of this article was published on November 26, 2014, at NEJM.org.

N Engl J Med 2017;376:928-38.
DOI: 10.1056/NEJMoa1410863
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ABSTRACT

BACKGROUND

The unprecedented 2014 epidemic of Ebola virus disease (EVD) prompted an international response to accelerate the availability of a preventive vaccine. A replication-defective recombinant chimpanzee adenovirus type 3–vectored ebolavirus vaccine (cAd3-EBO), encoding the glycoprotein from Zaire and Sudan species, that offers protection in the nonhuman primate model, was rapidly advanced into phase 1 clinical evaluation.

METHODS

We conducted a phase 1, dose-escalation, open-label trial of cAd3-EBO. Twenty healthy adults, in sequentially enrolled groups of 10 each, received vaccination intramuscularly in doses of 2×10^{10} particle units or 2×10^{11} particle units. Primary and secondary end points related to safety and immunogenicity were assessed throughout the first 8 weeks after vaccination; in addition, longer-term vaccine durability was assessed at 48 weeks after vaccination.

RESULTS

In this small study, no safety concerns were identified; however, transient fever developed within 1 day after vaccination in two participants who had received the 2×10^{11} particle-unit dose. Glycoprotein-specific antibodies were induced in all 20 participants; the titers were of greater magnitude in the group that received the 2×10^{11} particle-unit dose than in the group that received the 2×10^{10} particle-unit dose (geometric mean titer against the Zaire antigen at week 4, 2037 vs. 331; P=0.001). Glycoprotein-specific T-cell responses were more frequent among those who received the 2×10^{11} particle-unit dose than among those who received the 2×10^{10} particle-unit dose, with a CD4 response in 10 of 10 participants versus 3 of 10 participants (P=0.004) and a CD8 response in 7 of 10 participants versus 2 of 10 participants (P=0.07) at week 4. Assessment of the durability of the antibody response showed that titers remained high at week 48, with the highest titers in those who received the 2×10^{11} particle-unit dose.

CONCLUSIONS

Reactogenicity and immune responses to cAd3-EBO vaccine were dose-dependent. At the 2×10^{11} particle-unit dose, glycoprotein Zaire–specific antibody responses were in the range reported to be associated with vaccine-induced protective immunity in challenge studies involving nonhuman primates, and responses were sustained to week 48. Phase 2 studies and efficacy trials assessing cAd3-EBO are in progress. (Funded by the Intramural Research Program of the National Institutes of Health; VRC 207 ClinicalTrials.gov number, NCT02231866.)

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In August 2014, the World Health Organization declared the epidemic of Ebola virus disease (EVD) in West Africa to be a public health emergency of international concern. This was the first time in more than 20 outbreaks since EVD was identified in 1976 that such a declaration had been made.[1] The epidemic accounted for more cases of EVD than all previous EVD outbreaks combined, with 28,616 cases and 11,310 deaths reported.[2,3] The majority of cases occurred in Guinea, Liberia, and Sierra Leone, with a small number of cases in other West African countries,[4] Europe, and the United States.[5] Whereas previous EVD outbreaks had been controlled by implementation of strategies for the identification of cases, isolation of case patients, and contact tracing, the scope and duration of this epidemic illustrated the importance of establishing additional prevention tools such as an effective vaccine.

Ebola virus is a filovirus with a 19-kb, nonsegmented, negative-strand RNA genome that encodes seven viral proteins.[6] The surface glycoprotein mediates viral entry into host cells[7,8] and has been the primary antigenic target for vaccine development.[9] We previously evaluated a series of gene-based approaches to developing an Ebola vaccine in Ebola challenge studies involving nonhuman primates,[10-12] and three early-generation candidate Ebola vaccines were assessed in clinical trials between 2003 and 2009.[13-15] Because of theoretical safety concerns involving wild-type Ebola glycoprotein cytopathic effects in cell culture,[7] initial antigen designs included a transmembrane-deleted version of glycoprotein[13] and a full-length glycoprotein antigen containing a single amino acid mutation.[14] No safety concerns were identified with these vaccines, but advanced development was not pursued because immunogenicity and subsequent preclinical efficacy results were deemed to be inadequate. Therefore, full-length wild-type glycoprotein delivered by DNA vaccination was evaluated clinically and was shown to be immunogenic.[15] These data supported the accelerated development of the chimpanzee adenovirus type 3 (cAd3) vaccine vector described here, which encodes wild-type glycoprotein antigens from the Zaire and Sudan species of ebolavirus.

The cAd3 Ebola vaccine (cAd3-EBO) was chosen for clinical development on the basis of a demonstration of significant efficacy in a nonhuman primate challenge model 5 weeks after a single injection and of partial efficacy at 10 months and low levels of preexisting antivector antibody in the human population. In addition, cAd3-EBO primes nonhuman primates for a boost with recombinant modified vaccinia Ankara (MVA) wild-type glycoprotein vaccine, which results in more durable protection from challenge at 10 months.[16]

Clinical development of the cAd3-EBO vaccine began in 2011; a Pre-Investigational New Drug Application (pre-IND) was submitted to the Food and Drug Administration (FDA) in 2013, and a phase 1 clinical trial was scheduled for the first quarter of 2015. However, in response to the emerging outbreak in May 2014, we accelerated the initiation of the cAd3-EBO phase 1 clinical trial, working closely with the FDA and condensing standard timelines. In November 2014, we reported preliminary safety and immunogenicity results of the vaccine. Here we report updated results, including results regarding the long-term durability of the cAd3-EBO vaccine in healthy adults.

Methods

Study Design and Participants

VRC 207 was a phase 1, dose-escalation, open-label, clinical trial designed to determine the safety, side-effect profile, and immunogenicity of an investigational recombinant cAd3 ebolavirus vaccine. Eligible participants were healthy adults, 18 to 50 years of age. Full details of the inclusion and exclusion criteria and study conduct can be found in the protocol, available with the full text of this article at NEJM.org.[17] Participants were recruited from the Washington, D.C., metropolitan area. The trial was conducted at the National Institutes of Health (NIH) Clinical Center by investigators at the Vaccine Research Center (VRC) of the NIH National Institute of Allergy and Infectious Diseases (NIAID). The study was reviewed and approved by the institutional review board at the NIAID. The Department of Health and Human Services guidelines for the protection of human research subjects were followed. All participants provided written informed consent before enrollment. VRC developed the vaccine, and members of that center performed the study.

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VACCINE

The cAd3 drug substances were manufactured at Advent, a subsidiary of Okairos (now GlaxoSmithKline), and the drug product (recombinant chimpanzee adenovirus type 3–vectored Ebola vaccine, VRC-EBOADC069-00-VP) was manufactured at the VRC Vaccine Pilot Plant, under contract with the Vaccine Clinical Materials Program, Leidos Biomedical Research. The vaccine is a sterile, aqueous, buffered solution that includes cAd3-EBO glycoprotein Zaire and cAd3-EBO glycoprotein Sudan drug substances, in a 1:1 ratio, in single-dose vials of 1×10^{11} particle units per milliliter of each drug substance (2×10^{11} particle units per milliliter total). Zaire and Sudan species were chosen because, of the five species of ebolavirus, these two species are responsible for the majority of EVD outbreaks.[2,18] VRC-DILADC065-00-VP was the formulation buffer in vaccine production and the diluent for the cAd3-EBO.

STUDY PROCEDURES

A single dose of vaccine was administered intramuscularly (in the deltoid muscle) by needle and syringe at a dose of 2×10^{10} particle units in group 1 and 2×10^{11} particle units in group 2. The dose-escalation plan specified that for the first three participants in each group, no more than one participant per day would be vaccinated, with interim safety reviews required before additional vaccinations were performed in that group. (Enrollment in group 2 could begin only after this process had been completed for the first three participants in group 1.) Safety monitoring included laboratory and clinical evaluations that were performed during protocol-specified study visits. Local and systemic reactogenicity and medication use for relief of symptoms were recorded by all participants for 7 days after vaccination through an electronic data-capture system (EMMES). Clinical assessments were performed, complete blood counts obtained, and levels of creatinine and alanine aminotransferase, prothrombin time, and activated partial-thromboplastin time (aPTT) measured at scheduled study visits during the first 4 weeks after vaccination. Adverse events were graded according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (modified from FDA Guidance, September 2007).[19]

ASSESSMENT OF EBOLA GLYCOPROTEIN-SPECIFIC ANTIBODY RESPONSES

Glycoprotein titers as assessed by enzyme-linked immunosorbent assay (ELISA) were measured as described previously.[12] A positive response was considered to be a significant increase in titer, expressed as the 90% effective concentration (EC90; the concentration at which there is a 90% decrease in antigen binding), over the baseline value ($P<0.05$). Glycoprotein ELISA titers were measured against the vaccine-matched Sudan species at weeks 0, 2, and 4, against Zaire (Mayinga) species at weeks 0, 2, 4, and 8, and against the newly discovered Zaire species Guinea strain (Zaire–Guinea) at weeks 0 and 4. ELISA titers were also measured against Zaire (Mayinga) for vaccine durability at weeks 1, 2, 4, 8, 16, 24, and 48.

ASSESSMENT OF EBOLA GLYCOPROTEIN-SPECIFIC T-CELL RESPONSES

Vaccine-induced T-cell responses were evaluated by means of a qualified intracellular cytokine staining assay, as described previously.[15] Cryopreserved peripheral-blood mononuclear cells obtained at weeks 0, 2, 4, and 8 were stimulated with overlapping peptide pools matching the vaccine inserts for glycoprotein Sudan and Zaire and were quantified to determine the proportion of total and memory CD4 and CD8 T cells producing interleukin-2, interferon-γ, or tumor necrosis factor (TNF). Participants were considered to have had a positive response if they had a positive CD4 or CD8 response to either peptide pool (measured by interleukin-2, interferon-γ, or TNF) at weeks 2, 4, and 8. To assess vaccine-induced glycoprotein-specific memory T-cell responses, memory CD4 and CD8 T cells were identified on the basis of CD45RA and CD28 expression, and their cytokine expression was quantified.

CAD3 AND AD5 SEROLOGIC ASSESSMENT

We performed an adenovirus serum neutralization assay to assess neutralizing antibody titers in order to determine baseline and vaccine-induced (week 4) neutralization of cAd3 and human Ad5. Reciprocal antibody titers are reported as the 90% inhibitory concentration (IC90; the titer at which 90% of infectivity is inhibited). The assay was performed according to previously described methods.[20]

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STATISTICAL ANALYSIS

Positive response rates with respect to the development of Ebola-specific antibodies and T-cell responses and adenovirus neutralization were calculated along with exact 95% confidence intervals. The antibody response (EC90) as assessed by ELISA is reported as the geometric mean titer with the 95% confidence interval. Fisher's exact test was used for between-group comparisons of positive response rates, Student's t-test for comparisons of the magnitude of the antibody response after log transformation, and the Wilcoxon test for comparisons of the magnitude of T-cell responses. The association of adenovirus neutralization with antibodies and T-cell responses was evaluated with the Spearman rank-correlation method. T-cell data from intracellular cytokine staining assays were analyzed and displayed with the use of SPICE, version 5.3.[21] Other analyses were performed with the R statistical package, version 3.1.1.

RESULTS

STUDY POPULATION

A total of 20 participants were enrolled and vaccinated from September 2 through September 23, 2014 (Fig. 1). The study population comprised 11 women and 9 men; the mean age was 37 years (range, 25 to 50) (Table 1). For assessment of the durability of response, an additional 17 participants who received the 2×10^{11} particle-unit dose were evaluated.

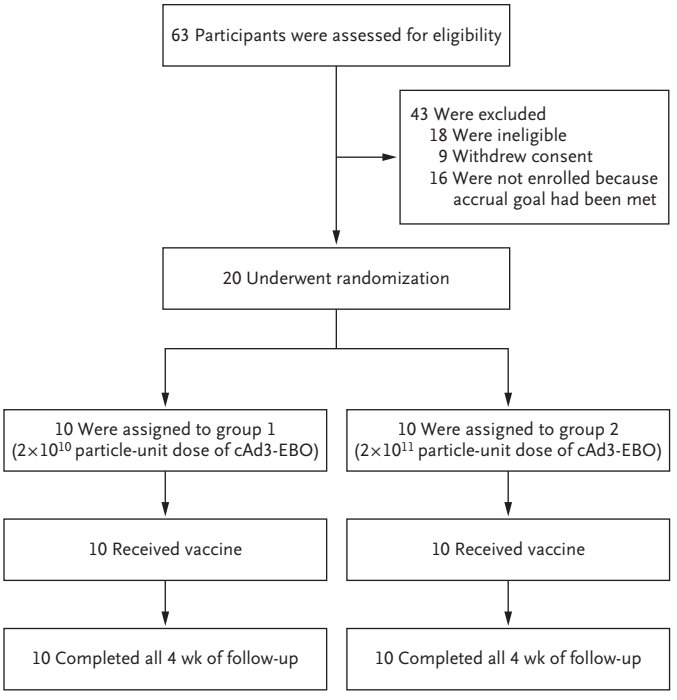

Figure 1. Screening, Enrollment, Vaccinations, and Follow-up.

Groups 1 and 2 were enrolled sequentially according to a dose-escalation protocol. All available study data and samples were used for study analyses. Overall, 100% of the participants completed the study-injection regimen and 4 weeks of follow-up. One participant was lost to follow-up at 48 weeks. For assessment of the durability of response at 48 weeks, an additional 17 participants who received the 2×10^{11} particle-unit dose were evaluated. The abbreviation cAd3 denotes chimpanzee adenovirus type 3.

VACCINE SAFETY

Information on local and systemic reactogenicity was solicited from participants at each scheduled visit. When present, reactogenicity was usually mild to moderate. There was evidence of a dose effect with respect to the use of medication for symptom relief (e.g., acetaminophen or nonsteroidal antiinflammatory drugs) and lowering of body temperature (Table S1 in the Supplementary Appendix, available at NEJM.org). No fever was reported after vaccination in the group that received the 2×10^{10} particle-unit dose; in the group that received the 2×10^{11} particle-unit dose, 2 of 10 vaccine recipients reported fever: one case of grade 1 fever (temperature of 38.1°C) and one of grade 3 fever (temperature of 39.9°C). Fever developed 8 to 24 hours after vaccination, responded to antipyretic medication, and resolved within 1 day. There were no serious adverse events.

Asymptomatic prolonged aPTT was observed 2 weeks after vaccination in one recipient of the 2×10^{10} particle-unit dose (aPTT, 70.5 seconds; grade 4) and in two recipients of the 2×10^{11} particle-unit dose (aPTT, 48.4 seconds and 58.4 seconds; grade 2 and grade 4, respectively). Further evaluation (Table S2 in the Supplementary Appendix) showed that all three instances of prolonged aPTT were consistent with the induction of an antiphospholipid antibody, which causes an in vitro effect in the laboratory assay for aPTT and does not indicate a coagulopathy. The aPTT measurement in these participants decreased by the next visit and returned to almost baseline levels before the end of the study.

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Table 1. Characteristics of the Participants at Enrollment.*

Characteristic	Group 1, 2×10^{10} Particle Units (N=10)	Group 2, 2×10^{11} Particle Units (N=10)	Overall (N=20)
Sex — no. (%)			
Male	3 (30)	6 (60)	9 (45)
Female	7 (70)	4 (40)	11 (55)
Age — yr	33.9±6.7	40.7±7.5	37.3±7.8
Race — no. (%)†			
Asian	1 (10)	1 (10)	2 (10)
Black	3 (30)	0	3 (15)
White	6 (60)	8 (80)	14 (70)
Multiracial	0	1 (10)	1 (5)
Hispanic or Latino ethnic group — no. (%)†	0	0	0
Body-mass index‡	26.8±5.5	27.0±3.5	26.9±4.5
College or higher educational level — no. (%)	10 (100)	10 (100)	20 (100)
cAd3 IC90 antibody titer >200 — no. (%)§	5 (50)	3 (30)	8 (40)

* Plus–minus values are means ±SD. The abbreviation cAd3 denotes chimpanzee adenovirus type 3.
† Race and ethnic group were reported by the participants.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters. This calculation was performed on the basis of weight and height measured at the time of screening.
§ The 90% inhibitory concentration (IC90) is the titer at which 90% of infectivity is inhibited.

Asymptomatic neutropenia or leukopenia of grade 1 or 2 was observed 3 to 4 days after vaccination in one recipient of the 2×10^{10} particle-unit dose and in three recipients of the 2×10^{11} particle-unit dose.

EBOLA-SPECIFIC ANTIBODY RESPONSES

At 4 weeks after vaccination, vaccine-induced anti-glycoprotein antibodies to Zaire species were detected in 90% of the participants in group 1 and in 100% of the participants in group 2, anti-glycoprotein antibodies to Sudan species were detected in 70% and 80% of the participants, respectively, and anti-glycoprotein antibodies to Zaire–Guinea species were detected in 90% and 90% of the participants, respectively. Anti-glycoprotein antibody responses (by ELISA) to at least one species or strain were detected in all 20 participants. At 8 weeks after vaccination, vaccine-induced anti-glycoprotein antibodies to Zaire–Mayinga species were detected in 90% of the participants in group 1 and in 80% of the participants in group 2. In both groups, the EC90 geometric mean titer against the Zaire–Mayinga antigen was higher at week 4 than at week 2 (331 vs. 106 in group 1 [P=0.001] and 2037 vs. 376 in group 2 [P<0.001]), as well as higher at week 4 than at week 8 (331 vs. 85 in group 1 [P=0.003], and 2037 vs. 464 in group 2 [P<0.001]) (Table 2 and Fig. 2). The EC90 geometric mean titer against the Sudan antigen was also higher at week 4 than at week 2 (279 vs. 161 in group 1 and 936 vs. 400 in group 2), and the difference reached significance in group 2 (P=0.004) (Table 2 and Fig. 2). The EC90 geometric mean titer against the Zaire–Guinea glycoprotein antigen was higher in group 2 than in group 1 at week 4 (623 vs. 177, P=0.02).

The durability of the vaccine-induced antibody response was assessed by ELISA. At 48 weeks after immunization, the latest time point sampled in this study, anti-glycoprotein antibodies to Zaire–Mayinga species remained elevated and similar to those at early time points. The high-dose group had consistently higher EC90 geometric mean titer values than the low-dose group. Across the time points sampled, both groups displayed the highest antibody responses at week 4 (Fig. 3 and Table 3), and titers remained high at week 48, which highlights the durability of the vaccine-induced antibody response.

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EBOLA-SPECIFIC T-CELL RESPONSES

Vaccine-induced CD4 and CD8 T-cell responses, as determined by expression of cytokines (interferon-γ, interleukin-2, and TNF) in response to stimulation with Zaire or Sudan glycoprotein peptides, were assessed by means of intracellular cytokine staining at weeks 2 and 4, and the expression of the cytokines (as a percentage of total CD4 and CD8 T cells) was compared with the baseline expression in all participants. Glycoprotein-specific CD8 responses to at least one antigen (Zaire or Sudan) were detected by week 4 in 20% of the participants in group 1 and in 70% of those in group 2 (P=0.07). Glycoprotein-specific CD8 responses to Zaire–Mayinga antigen were detected by week 8 in 10% of the participants in group 1 and in 40% of the participants in group 2. Glycoprotein-specific CD4 responses to at least one antigen (Zaire or Sudan) were detected by week 4 in 30% of the participants in group 1 and in 100% of those in group 2 (P=0.004). Glycoprotein-specific CD4 responses to Zaire–Mayinga antigen were detected by week 8 in 20% of the participants in group 1 and in 50% of the participants in group 2 (Table 2). Glycoprotein-specific memory CD4 and CD8 T-cell responses were greater in magnitude at week 4 than at week 2 and greater in group 2 than in group 1 (Fig. 4). The majority of the memory CD4 and CD8 glycoprotein-specific T-cell responses were polyfunctional, expressing two or three cytokines (Fig. 4); the vaccine elicited high proportions of CD8 cells coproducing interferon-γ and TNF, which are known to be associated with protection in nonhuman primates.[16]

CAD3 AND AD5 SEROLOGIC ASSESSMENT

Background immunity may affect the response to virus-vectored vaccines. We assessed cAd3 neutralizing antibody titers in all the participants at baseline and 4 weeks after vaccination and compared them with antibody and T-cell responses (Fig. S1 in the Supplementary Appendix). At baseline, reciprocal titers of anti-cAd3 neutralizing activity ranged from undetectable (<12) to 911. After vaccination, cAd3 antibody titers increased by a factor of at least 1.9 in all participants (Table S3 in the Supplementary Appendix). As assessed with the use of the Spearman rank-correlation method, preexisting antibodies to cAd3 did not significantly correlate with glycoprotein-specific antibody responses at week 4 for

Table 2. Geometric Mean Antibody Titers and Positive T-Cell Responses.*

Vaccine Group	Geometric Mean Antibody Titer (95% CI)					Percentage of Participants with Positive Response (95% CI)							
	Zaire Glycoprotein ELISA			Sudan Glycoprotein ELISA		Zaire Glycoprotein ELISA		Sudan Glycoprotein ELISA	CD4		CD8		
	week 2†	*week 4*†	*week 8*†	*week 2*	*week 4*‡	*week 4*	*week 8*	*week 4*	*week 4*§	*week 8*	*week 4*¶	*week 8*
Group 1: 2×10^{10} dose	106 (49–228)	331 (158–695)	85 (39-188)	161 (56–461)	279 (154–505)	90 (55–100)	90 (55-100)	70 (35–93)	30 (7–65)	20 (3-56)	20 (3-56)	10 (0-45)
Group 2: 2×10^{11} dose	376 (139–1023)	2037 (1007–4118)	464 (200-1080)	400 (132–1212)	936 (433–2021)	100 (69–100)	80 (44-97)	80 (44–97)	100 (69–100)	50 (19-81)	70 (35–93)	40 (12-74)

* Each vaccine group included 10 participants. Geometric mean titers were assessed with the use of glycoprotein enzyme-linked immunosorbent assays (ELISAs) at week 2, week 4, and week 8 for Zaire, and week 2 and week 4 for Sudan; positive responses with respect to glycoprotein ELISA and total glycoprotein-specific T-cells at week 4 and week 8 were assessed with the use of intracellular cytokine staining assays.

† Zaire glycoprotein ELISA titers were significantly higher in group 2 (high-dose group) than in group 1 (low-dose group) at week 2 (P=0.03), week 4 (P=0.001), and week 8 (P=0.004). Zaire glycoprotein ELISA titers were significantly higher at week 4 than at week 2 in both group 1 (P=0.001) and group 2 (P<0.001) and were significantly higher at week 4 than at week 8 in group 1 (P=0.003) and group 2 (P<0.001).

‡ Sudan glycoprotein ELISA titers were significantly higher in group 2 than in group 1 at week 4 (P=0.01). Sudan glycoprotein ELISA titers were significantly higher at week 4 than at week 2 in group 2 only (P=0.004).

§ CD4 and CD8 response rates at week 4 were higher in group 2 than in group 1 (P=0.004 for CD4, and P=0.07 for CD8).

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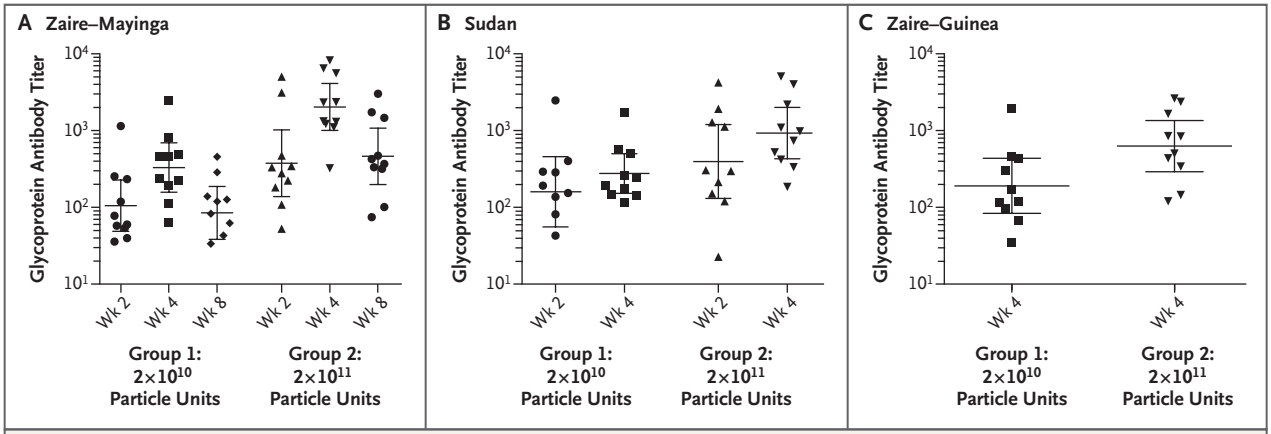

Figure 2. Glycoprotein Antibody Titers in Groups 1 and 2 as Assessed by an Enzyme-Linked Immunosorbent Assay (ELISA), According to Antigen.

Individual antibody titers as assessed by glycoprotein ELISA at 2 weeks and 4 weeks after vaccination, as well as at 8 weeks after vaccination for Zaire–Mayinga, are shown according to antigen and vaccine-dose group. Geometric mean titers (horizontal lines) are shown for each group and time point; I bars indicate 95% confidence intervals. Vaccine-induced antibodies against Zaire–Mayinga glycoprotein (Panel A) were higher at week 4 than at week 2 in both group 1 and group 2 (group 1, P=0.001, and group 2, P<0.001), as well as higher at week 4 than at week 8 (group 1, P=0.003, and group 2, P<0.001). The difference between the groups reached significance at weeks 2, 4, and 8 (P=0.03, P=0.001, and P=0.004, respectively). Vaccine-induced antibodies against Sudan glycoprotein (Panel B) were higher at week 4 than at week 2 in group 2 (P=0.004), and the difference between group 1 and group 2 reached significance at week 4 (P=0.01). Vaccine-induced antibodies against Zaire–Guinea glycoprotein (Panel C) were higher in group 2 than in group 1 at week 4 (P=0.02).

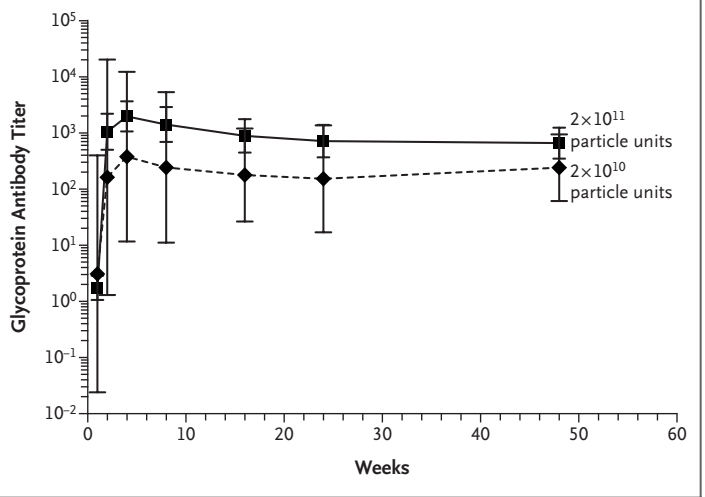

Figure 3. Durability of Glycoprotein Antibody Titers, as Assessed by an Enzyme-Linked Immunosorbent Assay (ELISA).

Individual vaccine-induced antibody titers against Zaire–Mayinga glycoprotein, as assessed by ELISA, at 1, 2, 4, 8, 16, 24, and 48 weeks after vaccination are shown according to vaccine-dose group. Geometric mean titers are shown for each dose group and time point. Peak titers were reached by week 4 in both dose groups, and within the high-dose group ($2x10^{11}$ particle units), week 4 titers were significantly higher than both week 1 titers (P<0.001) and week 48 titers (P<0.001).

Zaire species (correlation, −0.277; P=0.24) or Sudan species (correlation, −0.333; P=0.15). In addition, preexisting antibodies to cAd3 did not significantly correlate with the magnitude of vaccine-induced memory CD4 T-cell response (correlation, −0.232; P=0.33). There was a moderate association between the titer of preexisting antibodies to cAd3 and the memory CD8 T-cell response (correlation, −0.511; P=0.02) (Fig. S1 in the Supplementary Appendix).

Owing to the genetic relatedness of Ad5 to cAd3, anti-Ad5 neutralizing antibody titers were also assessed in all the participants at baseline and 4 weeks after vaccination. At baseline, reciprocal titers of anti-Ad5 neutralizing activity ranged from undetectable (<12) to higher than 8748 (Table S3 in the Supplementary Appendix). As assessed with the use of the Spearman rank-correlation method, preexisting antibodies to Ad5 did not significantly correlate with glycoprotein-specific antibody responses at week 4 for Zaire species (correlation, 0.194; P=0.41) or Sudan species (correlation, 0.162; P=0.50) or with vaccine-induced glycoprotein-specific memory T-cell responses for CD4 (correlation, −0.014; P=0.95) or CD8 (correlation, −0.144; P=0.55).

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Table 3. Durability of Vaccine-Induced Zaire–Mayinga Antibody Response*

Vaccine Group	Geometric Mean Antibody Titer (95% CI)			
	week 2	*week 4*	*week 8*	*week 48*
Group 1: 2×10^{10} dose	163 (1.3–20,334)	378 (12–12,294)	243 (11–5327)	241 (62–942)
Groups 2 and 5: 2×10^{11} dose†	1050 (501–2203)	1972 (1065–3653)	966 (338–2758)	659 (349–1245)

* Three participants were included in group 1 (low-dose group) and 18 participants in group 2 (high-dose group). Geometric mean titers were assessed with the use of Zaire glycoprotein ELISAs at weeks 2, 4, 8, and 48.

† For assessment of the durability of response, an additional 17 participants who received the 2×10^{11} particle-unit dose were evaluated (group 5). Within group 2, week 4 titers were significantly higher than both week 2 titers ($P<0.001$) and week 48 titers ($P<0.001$). Week 8 titers were also significantly higher than week 48 titers ($P=0.001$); however, no significant difference was observed between week 2 titers and week 48 titers.

DISCUSSION

These clinical data on the Ebola virus vaccine (cAd3-EBO) support the safety and immunogenicity of a single vaccination. The rates and severity of local and systemic side effects, including fever at higher dose levels, were similar to those observed in previous studies of other adenovirus vectors.[14,22] In future studies, a fever developing more than 1 day after vaccination or lasting longer than 1 day may require evaluation to determine additional causes. Vector-induced antiphospholipid antibodies were detected in 3 of 20 participants. This phenomenon was transient and has been reported previously with adenovirus vectors and licensed vaccines.[14,23-25] The antiphospholipid antibodies induced by vaccination bind the phospholipid reagent in the in vitro aPTT assay but do not have a clinical effect because phospholipids are not limited in vivo. The presence of these antibodies has not been shown to be associated with a clinical risk of coagulopathy or a hypercoagulable state. The observation of asymptomatic, mild-to-moderate neutropenia or leukopenia on day 3 to 4 after vaccination is consistent with margination (the relative adherence of leukocytes to endothelial cells temporarily preventing detection of the leukocytes in the bloodstream) related to vaccine-induced innate immune responses.

The relative contributions of antibodies and CD8 T cells to the protection of humans from EVD is unknown; however, both are thought to be important.[25] The vaccine-induced immune responses reported here are similar to those that are known to be associated with protection in efficacy studies of cAd3 and other adenovirus-vectored Ebola vaccines in nonhuman primates.[12,16,26] The geometric mean titer of the vaccine-induced glycoprotein Zaire–specific antibody at week 4 in group 2 was 2037, which was similar to reciprocal titers of 967 to 6600 in nonhuman primates protected by a 2×10^{10} particle-unit dose of cAd3-EBO.[16] The ELISA-binding antibody observed here is considered to be an indicator of "vaccine take" and is similar to that seen in protected nonhuman primates, as assessed with the same assay, standards, and controls; nonetheless, the antibody level is not thought to represent a mechanistic correlate of vaccine-induced protection.[27] Although the antibody titer that was observed here, as assessed with the glycoprotein ELISA assay, is associated with protection, anti-glycoprotein CD8 T-cell responses are also known to be important for protection from the high-dose challenge (median lethal dose, 1000 units) in nonhuman primates after vaccination.[25] In addition, we report durability of the antibody response in both dose groups at 48 weeks after vaccination, with little reduction in titers from the week 8 postvaccination time point. The glycoprotein-specific CD8 T-cell response at week 4 was detected in 70% of the participants who received the 2×10^{11} particle-unit dose and in 20% of those who received the 2×10^{10} particle-unit dose. Memory CD8 glycoprotein–specific T-cell responses were polyfunctional, and the responses followed a pattern that was similar to that seen in previous vaccine protection studies in nonhuman primates.[16,25]

A diminished immune response to some Ad5-based vaccines was previously reported in study participants who had preexisting neutralizing antibodies against Ad5.[14,27] Baseline titers of pre-

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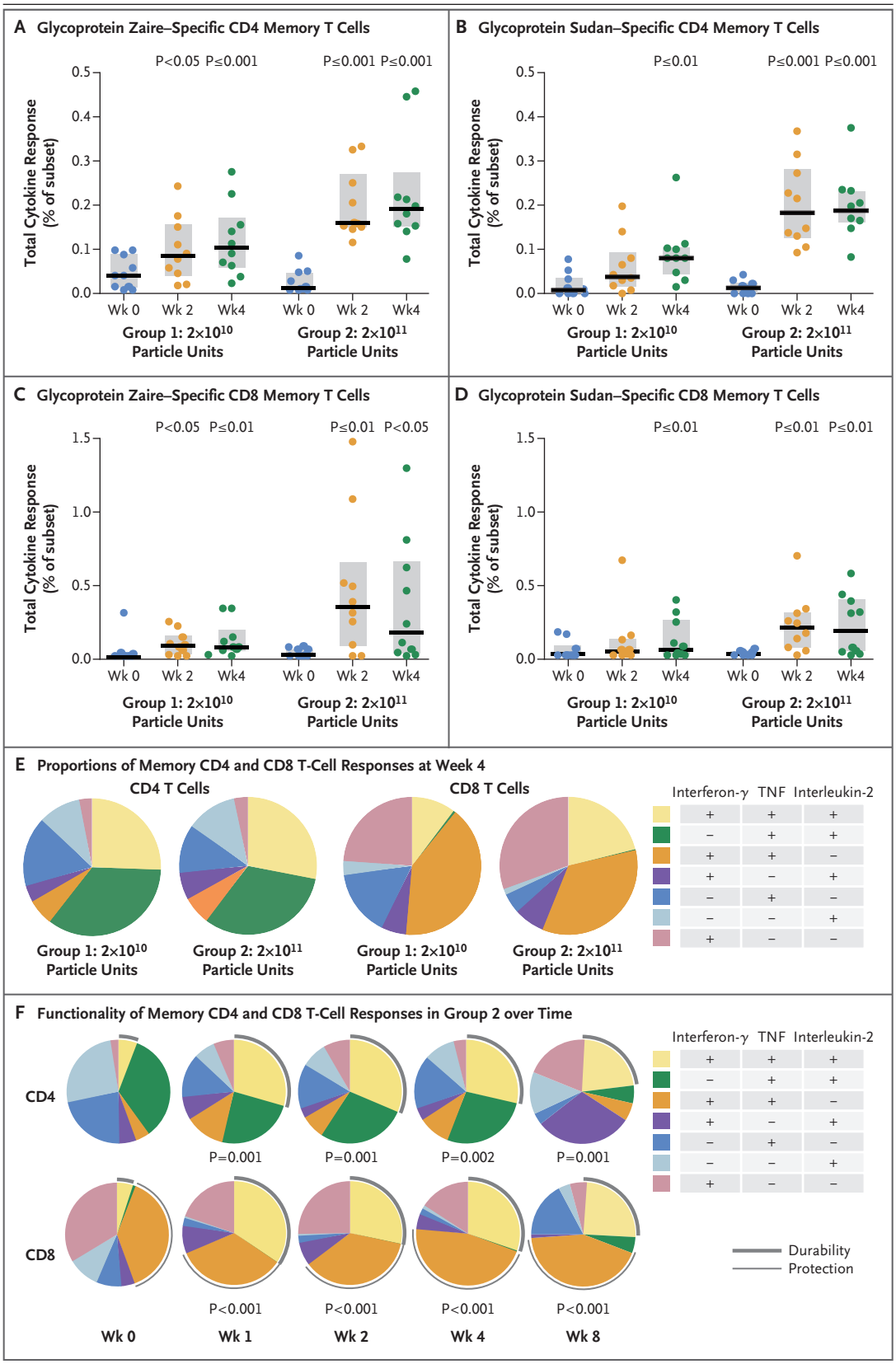

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Figure 4 (facing page). Vaccine-Induced Memory CD4 and CD8 T-Cell Responses, According to Dose.

Panels A through D show the individual responses for increases from baseline to week 2 and week 4 in glycoprotein Zaire–specific and glycoprotein Sudan–specific CD4 and CD8 memory T cells. A Wilcoxon test was used to calculate the P values for the comparison of the magnitude of the T-cell response; all comparisons are with week 0. Horizontal lines indicate medians, and shaded areas interquartile ranges. The proportions, at week 4, of glycoprotein-specific CD4 and CD8 T cells (i.e., cells secreting at least one cytokine) that make any given combination of the three cytokines are shown in Panel E. The proportion of vaccine-specific CD8 T cells capable of simultaneously secreting interferon-γ (interferon-γ+) and tumor necrosis factor (TNF+) is labeled; this level of cells with this functional profile was shown to be associated with protection in a preclinical nonhuman primate model. The kinetics of glycoprotein-specific CD4 and CD8 T cells making any given combination of the same three cytokines are shown in Panel F for group 2 at baseline, week 1, week 2, week 4, and week 8. Gray lines indicate profiles associated with protection (thin line) and durability (thick line) in a preclinical nonhuman primate model.

existing antibodies against cAd3 in our study participants were lower than those seen for Ad5 in the general population, a finding that was consistent with previous data.[28] The level of preexisting cAd3 antibodies did not significantly affect vaccine-induced glycoprotein-specific antibodies or CD4 T-cell immune responses. However, there was a moderate negative association between the titer of preexisting cAd3 antibodies and the memory CD8 T-cell response in participants with the highest baseline cAd3 antibody titers. Additional data are needed to understand the effect of preexisting cAd3 antibody titers on vaccine-induced immune responses. These data, coupled with the relatively low seroprevalence of cAd3 as compared with Ad5 worldwide, support the advanced development of this vaccine for the prevention of EVD.[28]

The bivalent vaccine reported here is composed of glycoprotein constructs from two Ebola species (Zaire and Sudan), but since the Zaire species of ebolavirus was responsible for the 2014 epidemic,[3] a monovalent cAd3-EBO Zaire vaccine (cAd3-EBOZ) was also prepared. This was done to increase manufacturing capacity and was supported by preclinical data in nonhuman primates that showed efficacy of the monovalent cAd3-EBOZ.[16,29] This vaccine was evaluated in a number of U.S. and international sites, with or without an MVA boost, to inform dosing, immunogenicity, and vaccine durability. In Mali (monovalent cAd3-EBOZ with MVA boost), Switzerland (monovalent cAd3-EBOZ without MVA boost), and the United Kingdom (monovalent cAd3-EBOZ with MVA boost), only mild adverse events were reported after a single vaccination with this vaccine. Placebo-controlled results in Switzerland showed that the types, severity, and rates of unsolicited events did not differ significantly between vaccine and placebo groups.[30] In all the studies, antibody responses peaked within 1 month after vaccination and were maintained at high levels through the remainder of the reporting period. Different doses of vaccine were also tested in these trials, with the highest dose and broadest range of doses tested in Mali. In this phase 1 Ebola vaccine study in a West African population, results identified $1x10^{11}$ particle units as the dose for additional trials, since it was not associated with clinically significant side effects and was more immunogenic than lower doses.[31] These results helped facilitate the initiation of phase 2 and 3 trials in West Africa (e.g., Pan African Clinical Trial Registry number, PACTR201504001092179 [phase 2 trial involving adults], and EudraCT number, 2014-004714-28 [phase 2 trial involving children]). Furthermore, when study sites in Mali and Oxford, United Kingdom, administered an MVA boost to a cohort of participants who were primed with the monovalent Zaire cAd3, results showed that ChAd3 antibody and T-cell responses were significantly increased after the MVA boost at the immediate postbooster time points, but this increase was smaller in the longer term.[31,32] It should be noted that longer-term, elevated antibody responses were achieved and maintained with a single administration of cAd3-EBO vaccine, and, as best shown in the Mali study,[31] durability did not require MVA boosting.

The data from the evaluation of the bivalent Zaire and Sudan vaccine with or without MVA boosting, as well as data from the ongoing efforts to further evaluate monovalent Zaire or Sudan cAd3 and Marburg cAd3 vaccines, provide an important foundation for the advancement of preventive filovirus vaccines.

The findings and conclusions in this report are those of the authors and do not necessarily reflect the views of the funding agency or collaborators.

Supported by the Intramural Research Program of the National Institutes of Health.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank the vaccine trial volunteers for their contribution and commitment to Ebola vaccine research; our colleagues at the National Institutes of Health (NIH) Clinical Center, including Khanh Nghiem, Theresa Doged, and Nilsa Almodovar; our colleagues at the National Institute of Allergy and Infectious Diseases (NIAID), including Anthony Fauci, Cliff Lane, Dean Follmann, Gregory Folkers, Hilary Marston, and Risa Eckes; the NIAID institutional review board (Koneti Rao and Doreen Chaitt); Richard Baumann and the NIH Institutional Biosafety Committee; current and previous colleagues at the NIAID Vaccine Research Center, including Hillery Harvey, Abraham Mittelman, Jason Gall, Robert Seder, Hope Wilson, and Gary Nabel; colleagues at the NIAID Office of Communications and Government Relations, Vaccine Pilot Plant, and Vaccine Clinical Materials Program, and Leidos Biomedical Research; personnel at EMMES, Iris De Ryck at GlaxoSmithKline, and Stefania DiMarco and Loredana Siani at Okairos.

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