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Baseline Asymptomatic Malaria Infection and Immunogenicity of Recombinant Vesicular Stomatitis Virus–Zaire Ebola Virus Envelope Glycoprotein Vaccine: The Sierra Leone Trial to Introduce a Vaccine Against Ebola (STRIVE)

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Background. The effect of malaria infection on the immunogenicity of the recombinant vesicular stomatitis virus–Zaire Ebola virus envelope glycoprotein (GP) vaccine (rVSVΔG-ZEBOV-GP) (ERVEBO) is unknown.

Methods. The Sierra Leone Trial to Introduce a Vaccine Against Ebola (STRIVE) vaccinated 7998 asymptomatic adults with rVSVΔG-ZEBOV-GP during the 2014–2016 Ebola epidemic. In STRIVE's immunogenicity substudy, participants provided blood samples at baseline and at 1, 6, and 9–12 months. Anti-GP binding and neutralizing antibodies were measured using validated assays. Baseline samples were tested for malaria parasites by polymerase chain reaction.

Results. Overall, 506 participants enrolled in the immunogenicity substudy and had ≥1 postvaccination antibody titer. Of 499 participants with a result, baseline malaria parasitemia was detected in 73 (14.6%). All GP enzyme-linked immunosorbent assay (ELISA) and plaque reduction neutralization test (PRNT) geometric mean titers (GMTs) at 1, 6, and 9–12 months were above baseline, and 94.1% of participants showed seroresponse by GP-ELISA (≥2-fold rise and ≥200 ELISA units/mL), while 81.5% showed seroresponse by PRNT (≥4-fold rise) at ≥1 postvaccination assessment. In participants with baseline malaria parasitemia, the PRNT seroresponse proportion was lower, while PRNT GMTs and GP-ELISA seroresponse and GMTs showed a trend toward lower responses at 6 and 9–12 months.

Conclusion. Asymptomatic adults with or without malaria parasitemia had robust immune responses to rVSVΔG-ZEBOV-GP, persisting for 9–12 months. Responses in those with malaria parasitemia were somewhat lower.

Keywords. Ebola; Ebola vaccine; immunogenicity; malaria; Sierra Leone.

The magnitude and complexity of the 2014–2016 Ebola outbreak led to concerns that conventional response measures would not control the outbreak. The global public health community, including affected countries recognized an urgent need for rapid development of safe and effective Ebola vaccines [1]. Several phase 1 studies of the candidate Ebola vaccine, recombinant vesicular stomatitis virus (VSV)–Zaire Ebola virus envelope glycoprotein (GP) vaccine (rVSVΔG-ZEBOV-GP; ERVEBO; Merck Sharp & Dohme, a subsidiary of Merck & Co) were conducted in Africa, Europe, and North America, and 3 phase 2/3 clinical trials were conducted in Africa, including the Centers for Disease Control and Prevention (CDC)–sponsored Sierra Leone Trial to introduce a Vaccine Against Ebola (STRIVE). These trials have provided data regarding the safety profile of this replication-competent recombinant vaccine and, in 1 trial in Guinea, demonstrated high vaccine efficacy when deployed for ring vaccination of contacts and contacts of contacts for Ebola cases [2–8]. This vaccine was deployed in subsequent Ebola outbreaks [9]. Since November 2019, it has been licensed in Europe, the United States, and several African countries and prequalified by the World Health Organization [10–13].

Although immunologic correlates of protection against Ebola have not been established, antibodies to *Zaire ebolavirus* GP are considered to be an indirect measure of protection [14, 15]. Challenge studies in nonhuman primates indicate that the humoral response to this GP is protective [16, 17]. Human trials have consistently documented a robust antibody response to the GP by 28 days after vaccination, with persistence for up to 2 years [2, 5–7, 18–22]. However, the titers required to prevent

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infection or severe outcomes remain unclear. In addition, direct comparison of human and nonhuman primate anti-GP titers is difficult, both because nonhuman primates mount higher humoral immune responses than humans and because of inherent difficulties with interpretation across studies, especially problematic for older studies that used unvalidated assays.

Understanding factors that may blunt rVSVΔG-ZEBOV-GP immunogenicity is important for widespread field implementation. Active malaria infection can inhibit the immune response to several vaccines [23–26]. Ebola outbreaks typically occur in African countries that include the countries with the highest malaria burden in the world [27], so evaluating the impact of malaria infection on rVSVΔG-ZEBOV-GP response has practical relevance. We report the results of the STRIVE immunogenicity substudy, in which validated assays were used to assess baseline seroprevalence of anti-GP and neutralizing antibodies and to document the magnitude and duration of antibody response through 9–12 months after vaccination with rVSVΔG-ZEBOV-GP. We also evaluate the association of baseline asymptomatic malaria infection with vaccine response.

MATERIALS AND METHODS

STRIVE was a clinical trial of rVSVΔG-ZEBOV-GP conducted during the 2014–2016 Ebola outbreak in Sierra Leone in which adult (≥18 years old), nonpregnant healthcare and frontline Ebola response workers were individually randomized to immediate or deferred (18–24 weeks later) vaccination and were followed up for 6 months to assess the safety and efficacy of the vaccine [8]. Detailed methods and safety results have been reported [8]. Potential participants with oral temperature >38°C or any acute illness symptoms were excluded. The rVSVΔG-ZEBOV-GP vaccine is a live attenuated replication-competent recombinant vaccine comprising an Indiana strain VSV vector in which the VSV envelope GP gene is replaced with that of the Kikwit strain of the Zaire Ebola virus. A total of 7998 participants received a single 1.0-mL nominal dose of 2×10^7 plaque-forming units/mL, given by intramuscular injection [8].

Immunogenicity Substudy Enrollment, Specimen Collection, and Handling

The immunogenicity substudy enrolled participants from 1 of the 7 STRIVE study sites (Connaught Hospital, Freetown) during June–September 2015, with a goal of accruing 500 substudy participants. Malaria transmission is perennial in Sierra Leone, with peak incidence during June and July. Substudy participants provided whole-blood samples collected in serum separator tubes just before vaccination (referred to as baseline) and at 1 month (±6 days), 6 months (±16 days), and 9–12 months (±20 days) after vaccination. The specimens were transported promptly to the STRIVE immunogenicity laboratory in Connaught Hospital, where they were centrifuged within an hour of arrival at 1100–1300*g* for 10 minutes to further separate serum and clot. Aliquoted serum samples from baseline and follow-up and blood clots from baseline specimens were stored in Sierra Leone at −80°C for up to 6 months, then shipped by air on dry ice to the CDC for longer-term storage. Aliquots of serum samples used in this study were sent to Sterigenics to be sterilized by treatment with gamma irradiation at the target dose of 50 kGy [28].

Malaria Testing

Malaria testing was performed at the CDC. Blood clots were homogenized in a 50-mL conical tube by passing them through a wire mesh and then centrifuging at 2000*g* for 5 minutes. From this homogenate, 200 µL was added directly to 20 µL of proteinase K in a 1.5-mL microcentrifuge tube, and DNA was extracted using the Qiagen DNA Mini Kit (Qiagen), according to the manufacturer's protocol. To assay for parasite DNA, real-time photoelectron-induced transfer polymerase chain reaction (PCR) was performed, as described elsewhere [29], with species-specific nested PCR used to confirm positive results [30]. To translate between the real-time photoelectron-induced transfer PCR result and estimated parasite density, cycle threshold values were extrapolated to parasite densities (in parasites per microliter of blood) through a DNA standard curve, as described elsewhere [29].

Antibody Measurement

All samples were tested at Q2 Solutions (formerly Focus Diagnostics). Total anti-GP immunoglobulin G was measured using the validated Filovirus Animal Nonclinical Group GP indirect enzyme-linked immunosorbent assay (ELISA) [31]. Microtiter plates were coated with purified ZEBOV Kikwit recombinant GP. Serum samples were incubated in the recombinant GP–coated wells with a serial-diluted reference standard and incubated with goat anti-human immunoglobulin G horseradish peroxidase conjugate. The enzymatic reaction was stopped using a sulfuric acid solution. Optical density was measured on an ELISA plate reader and titer concentrations (in GP-ELISA units per milliliter) were calculated from the standard curve, using a 4-parameter logistic curve fit.

Neutralizing antibodies to the rVSVΔG-ZEBOV-GP vaccine strain were measured using the validated plaque reduction neutralization test (PRNT). Serum was diluted from 1:5 to 1:10 240 and mixed with an equal volume of diluted rVSVΔG-ZEBOV-GP for final dilutions of 1:10 to 1:20 480. Neutralization proceeded over an 18-hour period at 2°C–8°C, after which the serum-virus mixture was used to inoculate Vero cells. Viral adsorption was done at 37°C ± 2°C for 60 minutes, followed by a methylcellulose overlay; cells were subsequently incubated at 37°C ± 2°C for 2 days. Plaques were visualized with crystal violet stain and counted using the ViruSpot from Autoimmun Diagnostika. A neutralizing titer was defined as one that resulted in a 60% reduction in viral plaques in the presence of serum, compared with virus control without serum.

Data Analysis and Interpretation

Age was dichotomized as 18–50 vs >50 years of age. Baseline malaria parasitemia was analyzed as present or absent. When malaria parasites were present, parasite density was categorized as "low" (<10/μL), "moderate" (10–200/μL), or "high" (>200/μL).

For both GP-ELISA and PRNT, analyses included calculation of geometric mean titer (GMT) at baseline and at 1, 6, and 9–12 months after vaccination, as well as geometric mean fold rise (GMFR) and seroresponse at 1, 6, and 9–12 months. For GP-ELISA, seroresponse was defined 2 ways: (1) ≥2-fold increase from baseline and ≥200 ELISA units (EU)/mL, the definition that best differentiated vaccine from placebo recipients in the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) clinical trial [32]; and (2) ≥4-fold increase from baseline. These definitions were prespecified in the development plan for the vaccine, though not specifically for this study. For PRNT, seroresponse was defined as ≥4-fold increase from baseline.

The 95% confidence intervals (CIs) for GMTs and GMFRs were based on analysis of variance, and the CI for seroresponse was based on the exact binomial method. For GMTs, all serum samples obtained at baseline or during the specified postvaccination window were included, whereas a baseline result was required for calculation of GMFRs and seroresponse. Comparisons between groups were made by assessing whether or not 95% CIs overlapped. Imputation was not performed for missing data. Baseline positive results were defined as ≥200 EU/mL for GP-ELISA and as any reaction above the lower limit of quantification (LLOQ) for PRNT; the LLOQ was 36.11 EU/mL for GP-ELISA, and 35 for PRNT. For measurements below the LLOQ, samples were assigned a result of half the LLOQ (eg, 18.06 EU/mL for ELISA).

These measures of vaccine immune response were calculated separately for participants in whom malaria parasitemia was present versus absent at baseline. In addition, analysis of covariance modeling of GP-ELISA and PRNT GMTs at each postvaccination time point by sex was performed, using baseline GP-ELISA and malaria parasitemia as covariates. Statistical analyses were conducted using SAS software, version 9.4.

Human Participants Protection

Trained STRIVE staff described this voluntary substudy to potential participants, and participants provided written informed consent to participate in the immunogenicity substudy; this consent was separate from and in addition to the informed consent to participate in the main STRIVE study. The immunogenicity protocol was reviewed and approved by the Sierra Leone Ethics and Review Committee, the Pharmacy Board of Sierra Leone, and the CDC Institutional Review Board. The study was conducted according to Good Clinical Laboratory Practice guidelines. STRIVE was registered with clinicaltrials.gov (NCT02378753) and the Pan African Clinical Trials Registry (PACTR201502001037220).

RESULTS

A total of 508 participants were vaccinated and enrolled in the immunogenicity substudy. For the overall GMT analysis, we excluded 2 participants who had no evaluable result from a sample obtained within the allowed postvaccination time windows, yielding 506 (99.6%) participants for analysis (Table 1). More than half of participants (293 of 506 [57.9%]) were men, and the large majority (468 of 506 [92.5%]) were 18–50 years of age. The subset of these participants who also had a baseline result were included in the overall GMFR and seroresponse analyses (503 of 506 [99.4%] for GP-ELISA and 438 of 506 [86.6%] for PRNT; Tables 1 and 2). For the malaria analyses, we included the 499 participants who had an evaluable result from the baseline malaria testing (Table 1).

Malaria Testing

Among the 499 participants with a baseline malaria result, parasitemia was detected in 73 (14.6%). Parasite density was low in 31 (42.5%), moderate in 29 (39.7%), and high in 13 (17.8%). The age group distributions of those with and those without malaria parasitemia were similar, but participants with parasitemia were more likely to be men (58 of 73 [79.4%]) than women (15 of 73 [20.6%]) (Table 1). However, among those with parasitemia, women were more likely than men to have high parasite density; men were the majority of the groups with low (93.6%) or moderate (69.0%) parasite density, whereas women were the majority (69.2%) of the high-parasite-density group (Table 1).

GP-ELISA and PRNT Measurement

Positive baseline GP-ELISA (≥200 EU/mL) titers were present in 76 of 506 participants (15.0%), and positive baseline PRNT titers were present in 4 of 438 (0.9%) (Table 1). All participants with positive baseline PRNT titers had positive baseline GP-ELISA results. The distribution of GP-ELISA results for the group with missing baseline PRNT results was similar to that of the group with an evaluable result (data not shown). GP-ELISA GMTs were higher than baseline (92.7 EU/mL) at 1, 6, and 9–12 months (964.3, 751.8, and 760.8 EU/mL, respectively; Table 2 and Figure 1A).

GMFRs showed a similar pattern, peaking 1 month after vaccination and persisting at 6 months and 9–12 months after vaccination (Table 2). Seroresponse, based on the definition of ≥2-fold increase in antibody titer from baseline and antibody titer ≥200 EU/mL, occurred in ≥87.8% of participants at each postvaccination assessment and in 94.1% at ≥1 postvaccination assessment (Table 2 and Figure 1B). Seroresponse based on the definition of ≥4-fold increase in titer was lower, occurring in 87.3% at ≥1 postvaccination assessment (Table 2). For PRNT, GMTs increased to 116 at 1 month and remained elevated, at

Table 1. Baseline Characteristics of Participants in the Immunogenicity Substudy of the Sierra Leone Trial to Introduce a Vaccine Against Ebola (STRIVE)

Characteristic	Participants, No. (%)						
					Parasite Density		
	Total (N = 506)	Any Baseline Malaria Result (n = 499)	Malaria Negative at Baseline (n = 426)	Malaria Positive at Baseline (n = 73)	Low (0.1 to <10/mL) (n = 31)	Moderate (10–200/mL) (n = 29)	High (>200/mL) (n = 13)
Sex							
Male	293 (57.9)	288 (57.7)	230 (54.0)	58 (79.4)	29 (93.6)	20 (69.0)	9 (30.8)
Female	213 (42.1)	211 (42.3)	196 (46.0)	15 (20.6)	2 (6.4)	9 (31.0)	4 (69.2)
Age							
18–50 y	468 (92.5)	461 (92.4)	392 (92.0)	69 (94.5)	30 (96.8)	28 (96.6)	11 (84.6)
>50 y	38 (7.5)	38 (7.6)	34 (8.0)	4 (5.5)	1 (3.2)	1 (3.4)	2 (15.4)
Positive for Ebola at baseline (before vaccination)							
GP-ELISA							
No	427 (84.4)	421 (84.4)	366 (85.9)	55 (75.3)	27 (87.1)	20 (69.0)	8 (61.5)
Yes	76 (15.0)	75 (15.0)	58 (13.6)	17 (23.3)	4 (12.9)	8 (27.6)	5 (38.5)
Missing[a]	3 (0.6)	3 (0.6)	2 (0.5)	1 (1.4)	0 (0)	1 (3.4)	0 (0)
PRNT							
No	434 (85.8)	427 (85.6)	365 (85.7)	62 (84.9)	27 (87.1)	24 (82.8)	11 (84.6)
Yes	4 (0.9)	4 (0.8)	3 (0.7)	1 (1.4)	0 (0)	1 (3.5)	0 (0)
Missing[a]	68 (13.4)	68 (13.6)	58 (13.6)	10 (13.7)	4 (12.9)	4 (13.8)	2 (15.4)

Note: Table includes participants with ≥1 evaluable result from a sample obtained within the allowed postvaccination time windows.

Abbreviations: GP-ELISA, glycoprotein enzyme-linked immunosorbent assay; PRNT, plaque reduction neutralization test.

[a]A baseline specimen was not available for 3 participants. An additional 65 participants had indeterminate results from baseline PRNT testing.

about 120, at 9–12 months (Table 2 and Figure 1C), and GMFRs and seroresponse measures also showed robust response. However, the seroresponse percentage was lower for PRNT than for GP-ELISA, with 81.5% meeting the PRNT seroresponse definition at ≥1 postvaccination assessment (Table 2 and Figure 1D).

Postvaccination GMTs were somewhat lower in men than in women, but no consistent pattern was seen by age group (Supplementary Figure 1). The baseline GMT was, by definition, higher in the baseline seropositive group. Postvaccination GMTs were also higher in this group, but the seroresponse percentages were nonetheless lower, because of the higher postvaccination GMTs required to meet either seroresponse definition from a high baseline GMT.

Association of Malaria Parasitemia With Immune Response

Baseline GP-ELISA titers were slightly higher in the group of participants in whom malaria parasitemia was present than in the group in whom it was absent (129 vs 87 EU/mL, respectively, with nonoverlapping CIs; Figure 1A and Supplementary Table 1). In the group of participants with parasitemia at baseline, postvaccination GP-ELISA GMTs and seroresponse percentages showed a trend toward lower responses at 6 and 9–12 months, although CIs overlapped widely (Figure 1B and Supplementary Table 1). PRNT analysis also showed lower point estimates for GMT titers and seroresponse percentages at each of the postvaccination time points in participants who were parasitemic at baseline, again with overlapping CIs (Figure 1C and 1D and Supplementary Table 2). However, the proportion meeting the PRNT seroresponse definition at *any* point after vaccination was significantly lower for parasitemic than for nonparasitemic participants (66.1% vs 84.3%, respectively; Figure 1D and Supplementary Table 2) with nonoverlapping CIs.

Analyses by categories of parasite density showed no consistent pattern of relationship between density and immune response (Supplementary Figure 2). Multivariate analyses of vaccine immune response including baseline malaria parasitemia (present vs absent) and baseline GP-ELISA as covariates yielded different results by sex so are presented separately (Figure 2). Among the group of participants who did not have baseline malaria parasitemia, this analysis confirmed the pattern of higher postvaccination GMTs in women. For the group of participants with malaria parasitemia, however, CIs were too broad to draw conclusions about differences by sex.

DISCUSSION

The STRIVE immunogenicity data demonstrate that vaccination with rVSVΔG-ZEBOV-GP elicited robust immune responses in vaccinated persons by 1 month after vaccination, responses that persisted, albeit at slightly lower GMTs, through 9–12 months after vaccination. These results are consistent with those other studies that reported immune responses to rVSVΔG-ZEBOV-GP, using the same validated ELISA and PRNT [22, 33]. The current study demonstrates that a somewhat lower response to vaccination is achieved in participants

Table 2. Overall Glycoprotein Enzyme-Linked Immunosorbent Assay and Plaque Reduction Neutralization Test Results at Baseline and 1, 6, and 9–12 Months After Vaccination with Recombinant Vesicular Stomatitis Virus–Zaire Ebola Virus Envelope Glycoprotein Vaccine, in the Immunogenicity Substudy of the Sierra Leone Trial to Introduce a Vaccine Against Ebola (STRIVE)

	Time Point				
Test and Result	Baseline	mo 1	mo 6	mo 9–12	Any Time After Vaccination
GP-ELISA (N = 506)[a]					
Participants in GMT analysis, no.	503	443	383	393	NA
GMT (95% CI)	92.7 (85.3–100.9)	964.3 (878.7–1058.3)	751.8 (690.6–818.4)	760.8 (697.6–829.8)	
Participants in GMFR analysis, no.	NA	441	381	393	NA
GMFR (95% CI)		10.7 (9.6–12.0)	8.1 (7.3–9.1)	8.4 (7.5–9.4)	
Seroresponders/participants in 1st seroresponse analysis, no.	NA	397/441	341/381	345/393	446/474
Seroresponse ≥2-fold increase from baseline and ≥200 EU/mL, % (95% CI)		90.0 (86.8–92.7)	89.5 (86.0–92.4)	87.8 (84.1–90.9)	94.1 (91.6–96.0)
Seroresponders/participants in 2nd seroresponse analysis, no.	NA	352/441	295/381	292/393	414/474
Seroresponse ≥4-fold increase from baseline, % (95% CI)		79.8 (75.8–83.5)	77.4 (72.9–81.5)	74.3 (69.7–78.6)	87.3 (84.0–92.0)
PRNT (N = 504)[a]					
Participants in GMT analysis, no.	438	437	382	396	NA
GMT (95% CI)	<35 (<35 to <35)	116.0 (105.7–127.4)	95.3 (86.3–105.3)	119.9 (107.9–133.2)	
Participants in GMFR analysis, no.	NA	376	326	342	NA
GMFR (95% CI)		6.3 (5.7–7.0)	5.4 (4.8–6.0)	6.8 (6.1–7.6)	
Seroresponders/participants in seroresponse analysis, no.	NA	265/376	211/326	237/342	334/410
Seroresponse ≥4-fold increase from baseline, % (95% CI)		70.5 (65.6–75.0)	64.7 (59.3–69.9)	69.3 (64.1–74.1)	81.5 (77.4–85.1)

Abbreviations: CI, confidence interval; EU, enzyme-linked immunosorbent assay units; GMFR, geometric mean fold rise; GMT, geometric mean titer; GP-ELISA, glycoprotein enzyme-linked immunosorbent assay; NA, not applicable; PRNT, plaque reduction neutralization test.

[a]Number of participants with serology data at ≥1 time point according to the study group to which they were randomized.

with asymptomatic malaria parasitemia at baseline. Although most participants with asymptomatic malaria infection met seroresponse definitions at most postvaccination time points, we found significantly lower PRNT seroresponse and trends toward lower GP-ELISA seroresponse and lower PRNT and GP-ELISA GMTs.

However, although the number of participants with high malaria parasite density was small, we saw no clear dose-response relationship. Thus, the clinical and practical relevance, if any, of these findings regarding asymptomatic malaria infection are unclear. It is plausible that malaria infection, especially symptomatic infection, could reduce the immune response to vaccination; this has been reported occasionally with vaccines against other infectious diseases [26]. However, our results should be interpreted in light of the clinical trial results from Guinea [4] and the vaccine effectiveness results from the 2018–2020 outbreak in the Democratic Republic of the Congo [9]. Although neither specifically assessed the impact of malaria infection on vaccine effectiveness, both showed that rVSVΔG-ZEBOV-GP provided excellent protection against Ebola in malaria-endemic settings.

Some complexities in our data that bear on the finding of lower vaccine response in participants with baseline malaria parasitemia merit further discussion. First, our observation that baseline GP-ELISA titers were higher in malaria-positive participants was unexpected. The lack of increased baseline PRNT seropositivity in malaria-positive participants argues against their having been exposed to Ebola, and no STRIVE participants had a clinical history of Ebola disease. Rather, we believe this finding is most likely attributable to nonspecific cross-reactivity due to the immune activation caused by malaria or to higher rates of previous non-Ebola infections that yielded cross-reactive antibodies. Perhaps baseline malaria infection helps explain the observation of higher baseline titers in other studies conducted in African populations, compared with non-African populations, although gamma irradiation of the STRIVE specimens certainly also contributed to the higher baseline titers we observed [22, 33]. STRIVE participants are likely to have had frequent malaria exposure throughout their lives; responses may differ in populations in which malaria is less common.

Second, seroresponse and GMFR, by definition, relate postvaccination titers to baseline titers. The lower seroresponse we observed in participants with baseline malaria parasitemia is thus to some extent attributable to higher baseline titers. However, this is not the whole story, as absolute postvaccination

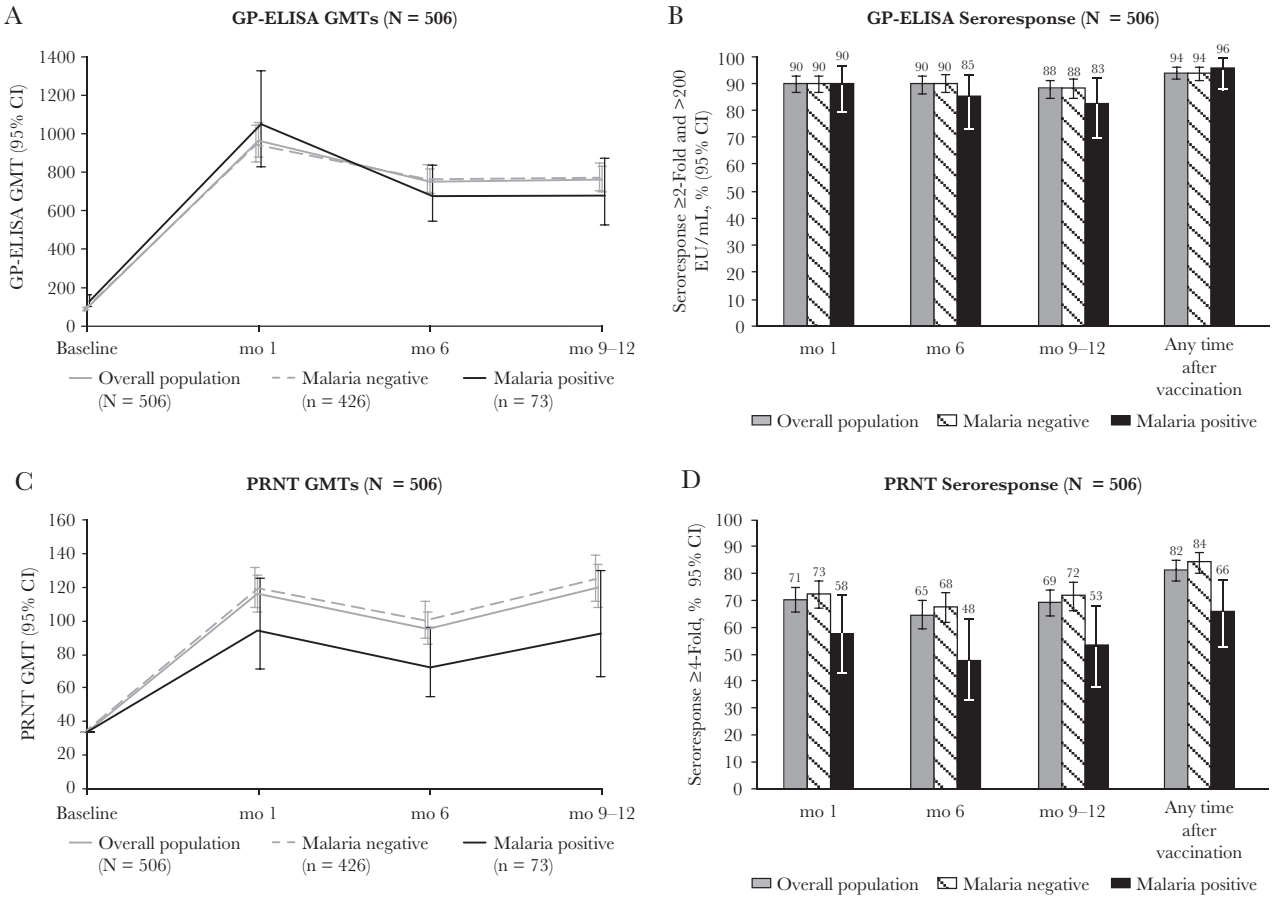

Figure 1. Antibody responses through months 9–12 after vaccination with recombinant vesicular stomatitis virus–Zaire Ebola virus envelope glycoprotein (GP) vaccine, by baseline asymptomatic malaria infection, in the immunogenicity substudy of the Sierra Leone Trial to Introduce a Vaccine against Ebola (STRIVE). *A, B,* GP enzyme-linked immunosorbent assay (ELISA) results. *C, D,* Plaque reduction neutralization test (PRNT) results, including geometric mean titers (GMTs) (*A, C*) and seroresponse percentages (*B, D*). A ≥2-fold increase from baseline along with ≥200 ELISA units (EU)/mL was the definition that best differentiated vaccine from placebo recipients in the Partnership for Research on Ebola Vaccines in Liberia (PREVAIL) clinical trial. Abbreviation: CI, confidence interval.

GMTs were also lower in participants with baseline malaria parasitemia. Third, further complicating the interpretation of the malaria data, is that women in our study were substantially less likely than men to have baseline malaria parasitemia—present in 7% of women versus 20% of men. Women appeared to have more vigorous responses to rVSVΔG-ZEBOV-GP than men, even in developed country settings where malaria is not endemic [20]. As our study included only 15 women with baseline malaria parasitemia, 4 of whom had a baseline positive GP-ELISA, it was not large enough to disentangle the effects of baseline GP-ELISA seropositivity, malaria parasitemia, and sex on vaccine response. It is also important to emphasize that this study was not designed or powered for hypothesis testing, so we have not conducted formal statistical testing.

Beyond our novel findings concerning the impact of asymptomatic baseline malaria infection, our results generally accord with the existing scientific literature on the immunogenicity of rVSVΔG-ZEBOV-GP. Robust immune responses have been demonstrated in separate studies in both regions considered to be at risk for Ebola Zaire outbreaks (African countries with previous outbreaks) and regions not at risk (North America and Europe) [2, 3, 5–7, 18, 19, 22]. Similarly to several previous studies, we found that a fraction of vaccinated persons—on the order of 5%–15%, depending on the postvaccination time point—did not produce anti-GP antibodies according to our criteria, though the implications for protection are unclear.

The impact of human immunodeficiency virus (HIV) infection on vaccine response is a concern, especially in high-prevalence areas. The trial in Liberia reported modest reductions in immune response with HIV infection [6]. STRIVE did not test participants for HIV infection, which is uncommon in adults in Sierra Leone, with an estimated prevalence of <2%, and no participants with self-reported HIV infection were included in the immunogenicity evaluation [34].

Our findings have several limitations beyond those already discussed. Regarding our malaria-related results, it is important to emphasize that the STRIVE participants were asymptomatic; our results cannot be generalized to persons with

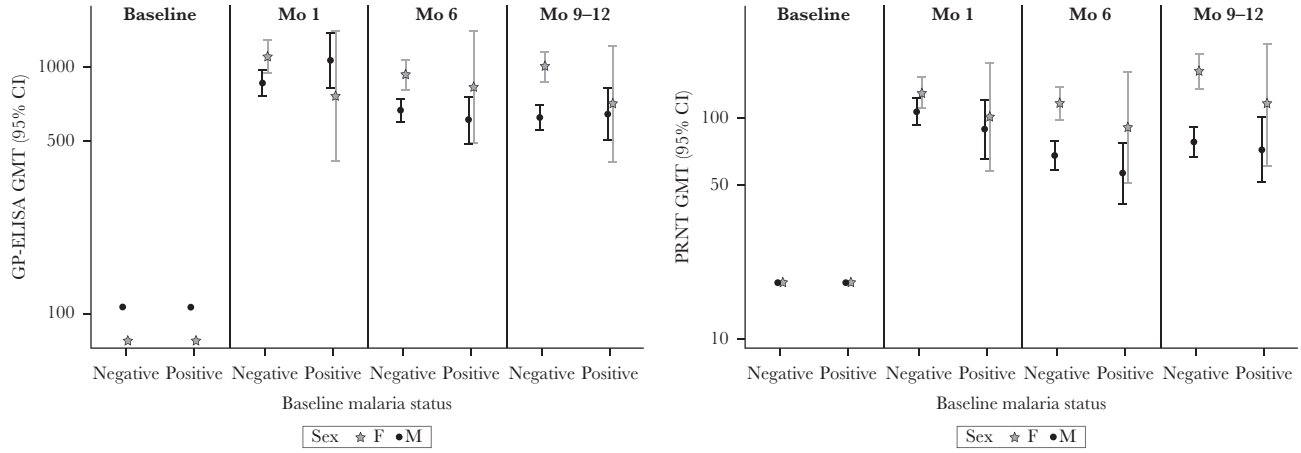

Figure 2. Antibody responses by baseline malaria parasitemia (present vs absent) and by sex, at baseline and 1, 6 and 9–12 months after vaccination with recombinant vesicular stomatitis virus–Zaire Ebola virus envelope glycoprotein (GP) vaccine. Results from the immunogenicity substudy of the Sierra Leone Trial to Introduce a Vaccine against Ebola (STRIVE) and based on analysis of covariance adjusted for the GP-ELISA GMTs and baseline malaria status by visit and sex. Abbreviations: CI, confidence interval; GMT, geometric mean titer; PRNT, plaque reduction neutralization test.

symptomatic malaria. Moreover, neither children nor pregnant women were included in STRIVE, yet both groups commonly experience malaria infection with high parasite density. Our results may not generalize to these groups, either. The differences we observed were generally small, with overlapping confidence limits, and their clinical implications for protection are unclear. It would not be surprising if any effects on vaccine immunogenicity were larger with symptomatic malaria, which is usually associated with higher parasite density.

We did not assess malaria infection after vaccination, but given the intensity of malaria transmission in Sierra Leone, it is likely that additional malaria infections occurred in many participants during follow-up. Our study provides no information about the possible impact of malaria infection during the postvaccination period on antibody durability. The use of validated assays is a strength of our study, but our results must be interpreted knowing that serum samples were gamma-irradiated to eliminate any theoretical risk of Ebola infection to laboratory workers. Gamma irradiation causes an approximate 20% elevation in prevaccination antibodies measured by the GP-ELISA, though not in those measured by the PRNT [28]. Irradiation also produces an approximate 20% reduction in postvaccination antibodies measured by both GP-ELISA and PRNT [35]. These changes are unlikely to change our general conclusions, since they are expected to affect all serum samples in the study similarly.

In conclusion, the STRIVE immunogenicity substudy reaffirms previous studies demonstrating robust immune response to rVSVΔG-ZEBOV-GP. Vaccination yields binding and functional antibody titers that remain elevated through 9–12 months after vaccination. Asymptomatic malaria infection may lower this response to some extent, highlighting knowledge gaps about the possible effects of malaria infection on vaccine-induced immune response and the importance of evaluating new vaccines in African populations, whose distinct exposure history to malaria and other infectious agents may have implications for vaccine response. More than 300 000 persons were vaccinated with rVSVΔG-ZEBOV-GP in the public health response to the second-largest Ebola outbreak in history, with 3470 cases and 2287 deaths, in 2018–2020 [36]. Many observers have credited the vaccine with helping prevent an even larger catastrophe [9]. Since then, rVSVΔG-ZEBOV-GP was again deployed in outbreaks in the Democratic Republic of the Congo and Guinea [37, 38]. Noting that the vaccine is being used for responses involving a wide range of participants with differing health status living in malaria-endemic areas, it is encouraging to know that most vaccinated persons, with or without asymptomatic malaria parasitemia, can be expected to have a strong immune response.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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