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Original article

Antibody responses to recombinant vesicular stomatitis virus-Zaire Ebolavirus vaccination for Ebola virus disease across doses and continents: 5-year durability

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ABSTRACT

Objectives: To report 5-year persistence and avidity of antibodies produced by the live-attenuated recombinant vesicular stomatitis virus (rVSV) expressing the Zaire Ebolavirus (ZEBOV) glycoprotein (GP), known as rVSV-ZEBOV (Ervebo®).

Methods: Healthy adults vaccinated with 300,000 or 10–50 million plaque-forming units of rVSV-ZEBOV in the WHO-coordinated trials of 2014–2015 were followed for up to 4 (Lambaréné, Gabon) and 5 (Geneva, Switzerland) years. We report seropositivity rates, geometric mean titres (GMTs), and population distribution of ZEBOV-GP ELISA IgG antibodies, neutralizing antibodies (pseudovirus and live-virus neutralization) and antibody avidity; the primary outcome was ZEBOV-GP ELISA IgG GMTs at 4 or 5 years compared with 1 year (Y1) after immunization.

Results: Among the 168 eligible vaccinees (Geneva: 97 and Lambaréné: 71) enrolled 1 year post-immunization, 146 (87%) remained enrolled at 4 years (Geneva: $n = 88$, Lambaréné: $n = 58$), and 84 (87%, Geneva) at 5 years post-vaccination. ZEBOV-GP ELISA IgG GMTs plateaued, with no declining trend from 1 year through the last time point assessed (1147.8 [95% CI 874.3–1507.0] at Y1 versus 1548.1 [95% CI 1136.6–2108.5] at Y5 in Geneva volunteers receiving $\geq$10 million plaque-forming units of rVSV-ZEBOV), their avidity matching that of ZEBOV convalescents. Live-virus neutralizing antibodies were detected for shorter periods and in fewer vaccinees (53/95 [56%] at Y1 versus 35/84 [42%] at Y5 in Geneva volunteers, all dose levels).

Discussion: Titres at Y1 emerged as a correlate of antibody persistence at Y5. The findings of persistent ZEBOV-GP ELISA IgG titres yet shorter-lasting, lower titres of live-virus neutralizing antibodies suggest the contribution of antibody-mediated protective mechanisms other than neutralization. Long-term

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clinical efficacy of rVSV-ZEBOV, however, requires further study. **Angela Huttner, Clin Microbiol Infect 2023;■:1**

Introduction

Ebolavirus (EBOV) disease (EVD) remains a significant global threat. Outbreaks have become larger and more frequent, appearing in new locations. Limited access to effective therapy, EBOV's ability to persist for years [1], its sexual transmission, and other factors make future epidemics inevitable unless preventive measures are implemented.

A live-attenuated recombinant vesicular stomatitis virus (rVSV) expressing the glycoprotein (GP) of Zaire Ebolavirus (ZEBOV), known as rVSV-ZEBOV (Ervebo®, Merck), was approved by the European Medicines Agency and the Food and Drug Administration and has been administered to >400 000 people. Short-term (<90 days) effectiveness of rVSV-ZEBOV approaches 100% [2]. Yet EVD cases have occurred among the immunized [3]; duration of protection has not been established. National/international bodies are thus questioned about the need for boosting, which is currently unapproved given insufficient data [4,5].

While immune correlates of protection have not been definitively established, humoral responses are key: anti-GP IgG antibodies (measured by ELISA) correlate with protection in nonhuman primates [6] and humans [7]; a cocktail of neutralizing human antibodies protected nonhuman primates [8]. Both neutralizing and binding antibodies associate with protection, the latter through Fc-mediated effector functions, albeit through unknown contributions [9].

Early antibody responses to rVSV-ZEBOV given at doses ≥10 million plaque-forming units (pfu) are robust and nearly universal in adults [10,11]. Yet titres drop to approximately 50% of their peak 1 year post-vaccination [12]. Here we characterize antibody responses up to 5 years post-vaccination by total ZEBOV-GP-binding and ZEBOV-neutralizing antibodies (measured by pseudovirus and live-virus neutralization assays), and avidity.

Methods

Design and participants

The phase I trials following healthy adults in Geneva, Switzerland, and Lambaréné, Gabon, for 1 year after single-dose rVSV-ZEBOV vaccination have been described (clinicaltrials.gov [NCT02287480 and NCT02296983] and Pan-African Trials Registry [PACTR201411000919191]) [10,13]. The Lambaréné trial, in which rVSV-ZEBOV was administered at either 300 000 pfu ('low-dose'), 3 million pfu ('intermediate-dose'), or 20 million pfu ('high-dose', HD), was extended to 4 years post-vaccination. At the close of the 1-year Geneva trial, which randomized participants to rVSV-ZEBOV 300 000 pfu (low-dose), 10 million pfu (HD), or 50 million pfu (HD), consenting volunteers joined a prospective observational study lasting 5 years post-vaccination (NCT02933931). The follow-up population here includes vaccinees from the Geneva observational study and the Lambaréné trial who adhered to their studies' protocols (e.g. underwent no further rVSV-ZEBOV vaccination) and had no suspected/documented EBOV exposure (see study protocols in the Appendix).

Laboratory analyses

Serum samples were prepared and kept frozen at −10°C or colder at all times.

Binding ZEBOV-GP antibodies. ZEBOV-GP-specific antibodies were quantified in the Diagnostic Systems Division at the US Army Medical Research Institute for Infectious Diseases (USAMRIID) in Frederick, Maryland, USA. The Filovirus Animal Non-Clinical Group-approved ELISA using the homologous Zaire−Kikwit strain GP was used following USAMRIID's standard operating procedure (SOP AP-03-35; USAMRIID ELISA) (see [4,10,13,14] and Appendix). To facilitate comparisons across studies, including earlier studies completed at USAMRIID in the Nonclinical Studies Division, antibodies were expressed in endpoint titres. A sample was considered seronegative if the corrected absorbance was less than the SOP-established 0.2 absorbance unit cut-off. Given that the lowest sample dilution on the plate was 1:50, any sample with lower titres was arbitrarily assigned a value of 1:25 for statistical analyses. As early (D0-Y1 [Lambaréné] and D0-Y2 [Geneva]) and subsequent samples were processed in two distinct laboratories within USAMRIID before and after a COVID-19-related interruption, early Geneva samples were rerun to assess inter-lab variability. This indicated that individual differences fell within two dilutions (twofold), though with higher titres for samples assessed in 2020 versus 2017. User-to-user, inter-plate, and day-to-day variation was within the SOP-acceptable criteria of less than 20% coefficient of variability in the new lab compared with before 2020−2021 testing.

Avidity and epitope mapping of ZEBOV-GP IgG antibodies. ZEBOV-GP IgG antibody avidity in late (Lambaréné: Y4 and Geneva: Y5) samples was assessed using Bio Layer Interferometry, with purified biotinylated ZEBOV-GP (immobilized onto SAX biosensors) as ligand (Appendix). EVD convalescent plasma samples ($n = 4$) served as comparators (Appendix). Binding responses of ligand-bound analytes were studied in real-time using an Octet K2 System (Sartorius, USA). Curves were processed using a 1:1 global fitting curve fit model using the Octet data acquisition software (version 10.0, Sartorius, USA) to obtain on-rates (Ka) and off-rates (Kd). Avidity was determined from the equilibrium dissociation constant (KD) for each binding interaction (Appendix).

Antibody epitopes were mapped using nine ZEBOV-GP-specific monoclonal antibodies (MoAbs) targeting different tiers on ZEBOV-GP (Appendix), including MoAbs KZ52 and 5.1.10B3, targeting the GP1 base and chalice bowl, respectively, and having neutralizing properties. EVD convalescent plasma samples ($n = 4$) and the WHO reference standard were used as comparators. A cut-off value of 20% was set according to Bio Layer Interferometry competition assay protocol; all values with >20% blocking were considered for statistical comparisons (Appendix).

Neutralizing antibodies assessed by pseudovirion (PsV) neutralization. PsV titres were assessed at USAMRIID using a VSV-pseudovirion expressing luciferase and using the Ebola Zaire 95 glycoprotein ([10,13] and Appendix). Early (D0−Y2) samples were rerun to assess inter-assay variability, which was limited to one dilution for most samples. Titres ≥20 were experimentally defined

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as positive; lower titres were arbitrarily assigned a 50% lower value for statistical analyses.

A second PsV neutralization assay using a VSV-pseudovirion expressing luciferase and green fluorescent protein (GFP) was developed at Spiez Laboratory (Switzerland) using the Ebola Zaire Guinea 2014 glycoprotein [15,16], where all Geneva samples were assessed (Appendix). Titres ≥6 were experimentally defined as positive; lower titres were arbitrarily assigned a 50% lower value for statistical analyses.

Neutralizing antibodies against live pathogenic EBOV. Neutralizing capacity was assessed in Spiez by serum neutralisation test (SNT) using infectious EBOV (Zaire Mayinga) expressing GFP provided by Stephan Becker, University of Marburg. Neutralizing antibodies (NA) were assessed from Y1 through Y5 in sera of Geneva vaccinees with detectable NA at M6 (Appendix). Titres ≥8 were defined as positive; lower titres were arbitrarily assigned a 50% lower value for statistical analyses.

Study outcomes

The primary outcome was geometric mean titre (GMT) of ZEBOV-GP-specific ELISA IgG antibodies in the final 3 years of follow-up after immunization (Y3–Y5) compared with Y1 GMTs (see [12]). As antibodies are initially produced by short-lived and subsequently by long-lived plasma cells, persistence was defined by the ratio of anti–ZEBOV-IgG GMTs at later time points to Y1 GMTs, excluding the influence of short-lived plasma cells. GMTs and ratios to Y1 were used to characterize NA titres and persistence, respectively. Kd was the main outcome for antibody avidity.

Statistical analysis

Sample size. The size of the two follow-up cohorts was predetermined by the number of vaccinees fulfilling eligibility criteria and adhering to the studies' protocols.

Analyses. ZEBOV-GP-specific IgG GMTs, NA, and 95% CIs were calculated for all volunteers with available data after a logarithm base-10 transformation. Seropositivity rates were defined as titres above given thresholds. Comparisons of GMTs and seropositivity rates between independent groups were conducted using t-test and Fisher's exact test. Geometric mean fold differences (ratio) between time points were assessed. Associations between strong ZEBOV-GP-binding antibody responses (defined by a titre in the highest quartile) at Y4 (Lambaréné) or Y5 (Geneva) and demographic, clinical, and immunological factors were investigated by univariate and multivariate regression models. For avidity analysis, Ka and Kd values in vaccinees and EVD convalescents were normalized against Ka and Kd values from the WHO reference standard (NIBSC, UK) using the formula: $yN, i = yWHOi \sum WHOim$. Wilcoxon signed-rank test was used for comparisons of binding associations between groups; statistical significance was denoted as * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$ using prism version 9.4.1. More details are provided in the Appendix.

Results

Demographic, clinical, and immunologic characteristics

The baseline (Y1 post-vaccination) population consisted of 168 volunteers: 97 in Geneva and 71 in Lambaréné. Table S1 summarises their dose groups and other characteristics; Fig. S1 details study flow. Participation remained high for all years of observation (Fig. S1). Twenty-three Geneva vaccinees had experienced transient vaccine-induced arthritis [10,13]; all fully resolved without relapse. No participant reported Ebolavirus exposure.

ZEBOV-GP-binding IgG persistence

Seropositivity persisted in all volunteers, with titres ≥50 at Y4 and Y5 in 58 of 58 and 83 of 83 Lambaréné and Geneva volunteers, respectively (Table S2).

As previously described (and included in Table 1 and Fig. 1 to facilitate comparisons), ZEBOV-GP-binding IgG ELISA responses peaked between D28 and M6 in a dose-dependent manner, declining thereafter [10,13]. ELISA GMTs increased (approximately twofold—e.g. one dilution) between Y2 and Y3 (Geneva) and

Table 1
Geometric mean titres of ZEBOV-GP-specific antibodies as measured by ELISA across doses and time points (USAMRIID laboratory)

	Doses	*N* Day 0 GMT 95% CI	*N* Day 28 GMT 95% CI	*N* Month 6 GMT 95% CI	*N* Year 1 GMT 95% CI	*N* Year 2 GMT 95% CI	*N* Year 3 GMT 95% CI	*N* Year 4 GMT 95% CI	*N* Year 5 GMT 95% CI
Geneva	300 000 pfu	*48* *26.1* *24.5–27.8*	*48* *351.3* *227.9–541.5*	*48* *1022.6* *712.9–1466.8*	*48* *800.0* *545.6–1173.1*	*46* *493.8* *334.8–728.5*	43 924.9 627.3–1363.7	40 1037.5 701.7–1534.0	41 915.9 611.4–1371.9
	10 million pfu	*34* *33.9* *26.3–43.8*	*34* *1064.3* *747.8–1514.5*	*33* *1634.0* *1177.1–2268.1*	*33* *1143.3* *794.4–1645.5*	*31* *874.8* *590.9–1295.3*	29 1600.0 1132.9–2259.7	30 1637.4 1155.1–2323.1	30 1600.0 1095.9–2336.0
	50 million pfu	*15* *31.5* *23.0–43.1*	*14* *1681.2* *965.9–2926.2*	*15* *1837.9* *1107.2–3050.7*	*15* *1157.8* *770.8–1739.3*	*14* *767.8* *467.6–1260.6*	12 1510.2 973.9–2341.9	12 1600.0 973.5–2629.6	12 1425.4 768.3–2644.7
	10 or 50 million pfu	*49* *33.2* *27.3–40.3*	*48* *1216.1* *907.4–1629.8*	*48* *1695.1* *1300.1–2210.2*	*48* *1147.8* *874.3–1507.0*	*45* *840.0* *621.8–1134.8*	41 1573.2 1206.2–2051.9	42 1626.6 1234.2–2143.8	42 1548.1 1136.6–2108.5
Lambaréné	300 000 pfu	*18* *42.9* *31.0–59.3*	*19* *960.1* *444.4–2074.1*	*15* *763.9* *372.7–1565.6*	*16* *610.4* *308.7–1206.7*	19 860.6 458.8–1614.0	17 1415.7 670.2–2991.0	17 1108.5 575.2–2136.5	ND
	3 million pfu	*37* *39.1* *29.7–51.8*	*37* *1351.8* *929.0–1966.9*	*35* *1286.8* *995.4–1663.5*	*36* *824.8* *641.7–1060.2*	32 1862.0 1498.1–2314.2	35 1874.7 1412.0–2489.0	29 1984.0 1482.5–2655.3	ND
	20 million pfu	*15* *75.8* *37.9–151.5*	*15* *1527.7* *833.1–2801.5*	*14* *2377.6* *1389.3–4068.8*	*13* *1363.5* *609.7–3049.3*	13 3755.1 2121.3–6647.3	12 2135.7 1591.0–2867.0	11 3865.9 2312.3–6463.4	ND

D0–Y2 (Geneva) and D0–Y1 (Lambaréné) GMTs (italics, grey zone) had been published but were recalculated for the follow-up cohorts described here to facilitate comparisons with subsequently assessed samples.
GMT, geometric mean titre; pfu, plaque-forming units; USAMRIID, US Army Medical Research Institute for Infectious Diseases; ZEBOV-GP, Zaire Ebolavirus glycoprotein.

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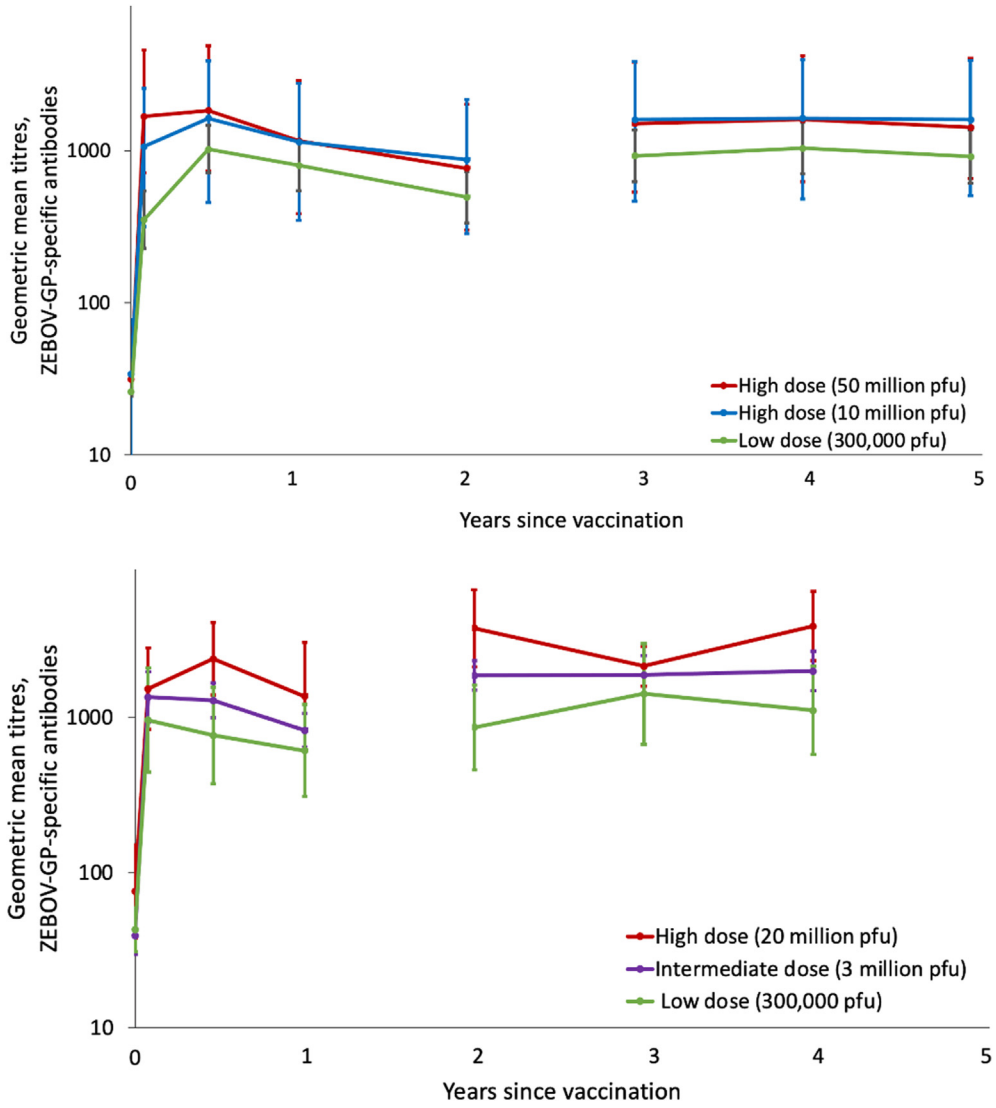

Fig. 1. Geometric mean titres of ZEBOV-GP-specific antibodies in Geneva (a) and Lambaréné (b) over time. Vertical bars represent 95% CIs. The left part of each graph indicates previously published data [12] but here includes only participants included in these follow-up cohorts; the right part represents novel antibody persistence data. ZEBOV-GP, Zaire Ebolavirus glycoprotein; pfu, plaque-forming units.

between Y1 and Y2 (Lambaréné), corresponding to the time at which early and late samples were assessed at USAMRIID. Samples drawn at Y2–4 in Lambaréné and Y3–5 in Geneva, measured simultaneously, revealed stable GMTs regardless of vaccine dose (Fig. 1, Table 1): GMT ratios were close to or above 1.0 compared with Y1 (Table S3).

Reverse cumulative distribution curves (RCD) show that in Geneva vaccinees, Y3–5 titre distribution was unchanged compared with Y1, regardless of vaccine dose (Fig. S2). In Lambaréné, RCDs for Y2–3–4 samples (assessed simultaneously) were also overlapping. Thus, one dose of rVSV-ZEBOV generated sustained specific IgG responses, plateauing through last time point assessed.

ZEBOV-GP-binding IgG avidity

In the Geneva cohort, binding kinetics of ZEBOV-GP-binding IgG in Y5 vaccinees ($n = 83$) had high on-rates and better off-rates and were comparable to that of EVD convalescents and WHO reference standard (Fig. 2a–c); avidity was 10^{-11} to 10^{-4} M, with no statistically significant difference among dose groups.

Antibodies at Y4 from Lambaréné vaccinees ($n = 56$) similarly showed sustained high on-rates, better off-rates, and high avidity (10^{-12} to 10^{-7} M), with no significant differences in binding kinetics nor avidity among dose groups (Fig. 2d–f).

Epitope targeting of ZEBOV-GP-binding IgG

Vaccinees' antibodies blocking the epitopes (y-axis) of selected MoAbs (x-axis) on ZEBOV-GP were plotted (Fig. 3). Although negative-control plasma IgG antibodies blocked no MoAbs over the 20% threshold, Geneva vaccinees' antibodies blocked about 20–40% of c-terminus, 20–60% of chalice bowl, mucin-like domain and glycan cap regions, 20–30% of base of GP1, 20–30% of fusion loop and 20–40% of HR2 regions on ZEBOV-GP. Lower blocking (20–35%) to mucin-like domain on the outer membrane region was observed (Fig. 3). The overall profile in Lambaréné was comparable except for higher blocking (20–50%) to the GP1 base targeted by neutralizing KZ52 MoAb. Fewer Geneva samples significantly blocked epitopes on different tiers of GP (Fig. 3). EVD convalescent plasma and the WHO reference standard showed a statistically

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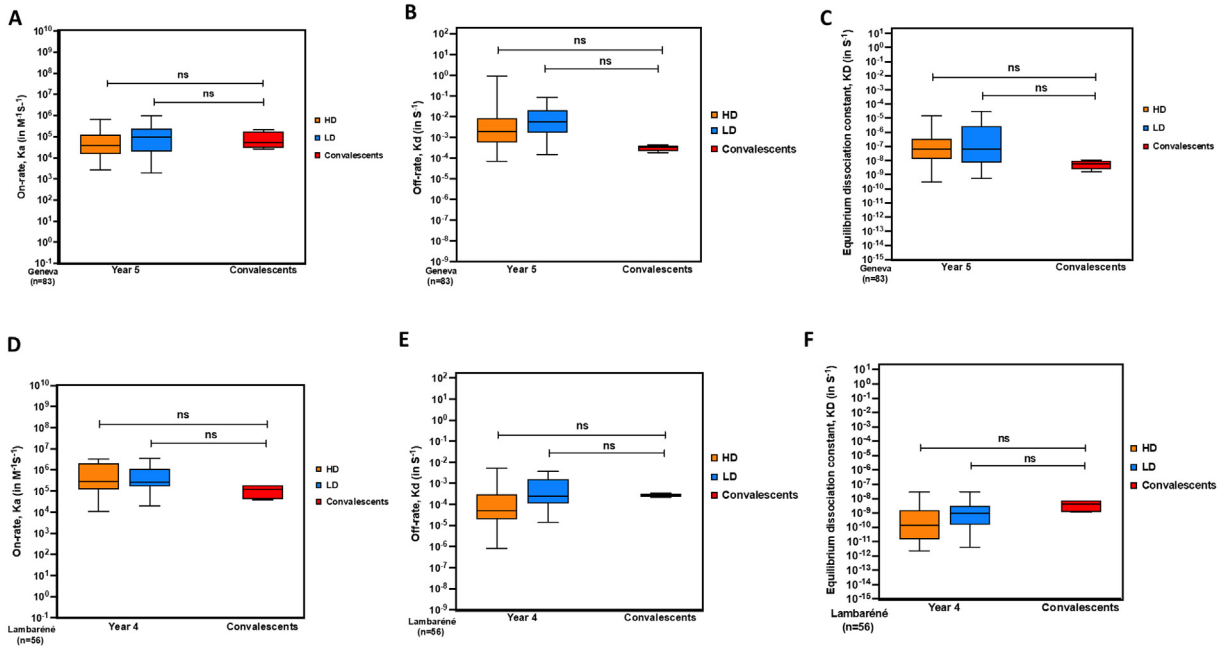
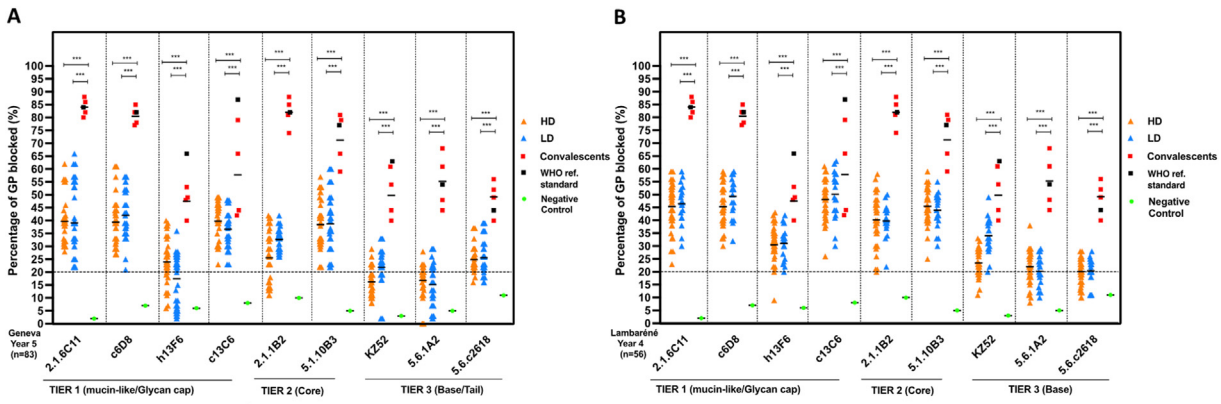


Fig. 2. High avidity ZEBOV-GP specific IgG antibodies in vaccinees in Geneva (a–c) and Lambaréné (d–f) vaccinees. Shaded boxes represent the interquartile range, with the median marked as the horizontal line. Full range is noted by the upper and lower bars. On-rates; Ka (a), off-rates; Kd (b) and equilibrium dissociation constants; KD (c) of purified IgG antibodies from Geneva vaccinees ($n = 83$) 5 years after a high (HD) or a low (LD) vaccine dose to the immobilized ZEBOV-GP purified protein are shown in box plots. Binding kinetics between two vaccine groups were compared with antibodies from EVD convalescents ($n = 4$). Similarly, Ka (d), Kd (e), and KD (f) of antibodies from Lambaréné vaccinees ($n = 56$) 4 years after a high (HD) or a low (LD) vaccine dose were plotted. Wilcoxon signed-rank test was used for comparisons between groups and statistical significance was denoted as * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$. EVD, Ebolavirus disease; ZEBOV-GP, Zaire Ebolavirus glycoprotein.



C

ZEBOV-GP-specific MoAbs	Tiers on ZEBOV-GP	Known Epitopes on ZEBOV-GP; Function
2.1.6C11 (commercial)	1	Mucin rich domain; virus entry and attachment
c6D8 (commercial)	1	Mucin rich domain; virus entry and attachment
h13F6 (commercial)	1	Mucin rich domain; virus entry and attachment
c13C6 (commercial)	1	Glycan cap; virus entry and attachment
2.1.1B2 (Isolated	2	C-terminus; Protective
5.1.10B3 (Isolated)	2	Chalice bowl; neutralizing
5.6.1A2 (Isolated)	3	Fusion loop; conserved region
KZ52 (commercial)	3	Base; neutralizing
5.6.c2618 (Isolated)	3	HR2; conserved region

Fig. 3. ZEBOV-GP-specific IgG antibodies targeting epitopes on regions vital for EBOV attachment, entry, and neutralization. MoAbs that target different tiers on ZEBOV-GP (c) were used to map targeting epitopes of antibodies from Geneva (a) and Lambaréné (b) vaccinees (and of EVD convalescent, WHO reference and negative controls) on ZEBOV-GP. Wilcoxon signed-rank test was used for comparisons between vaccine dose groups and EVD convalescents where statistical significance was denoted as * for $p < 0.05$, ** for $p < 0.01$, and *** for $p < 0.001$. The dashed horizontal line represents the 20% positivity threshold. EBOV; Ebolavirus; EVD, Ebolavirus disease; MoAbs, monoclonal antibodies; ZEBOV-GP, Zaire Ebolavirus glycoprotein.

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significantly higher blocking (range 40–90%) than vaccinees' sera (Fig. 3).

Neutralizing antibodies by pseudovirion assays

Neutralizing seropositivity rates measured by USAMRIID's PsV assay [10] in Geneva vaccinees at Y3, Y4, and Y5 were 71% (58/82), 74% (60/81), and 72% (60/83), respectively. In Lambaréné, seropositivity rates were 66% (42/64), 69% (44/64), and 56% (32/57) at Y2, 3, and 4, respectively. As described [10], PsV titres peaked on D28 and were significantly lower by M6. GMTs however subsequently remained stable between Y3–4 (Lambaréné) and Y3–5 (Geneva), independent of vaccine dose (Table 2, panel A).

PsV titres assessed by the Spiez assay were lower (seropositivity rates 56% [53/95] at Y1), but declined slowly (Y2: 53% [46/87]; Y3: 46% [39/84]; Y4: 46% [39/84]; Y5: 42% [35/84]). GMTs peaked between M6 and Y1, declined by Y3 and remained stable thereafter (Table 2, panel B, Fig. S3). The influence of dose was minimal, with the greatest variation observed between Y1 and Y3.

Both PsV assays found no significant decline of PsV seropositivity or titres during the last 3 years of follow-up.

Neutralizing antibodies by serum neutralization test using live EBOV

In Geneva vaccinees, NT measured by the SNT had peaked on D28 and rapidly declined by M6 and Y1 [10], reaching low or undetectable titres in all groups. Vaccinees with detectable NT at Y1 had titres persisting through Y5 regardless of dose (Tables S4 and S5; Fig. S4).

Determinants of persistent, high ZEBOV-IgG antibody titres

In Geneva, post-vaccination arthritis correlated with higher early ZEBOV-GP-specific antibody responses [10,13]. At Y5, arthritis, and strong (highest quartile) D28 and Y1 antibody titres were predictors of strong (highest quartile) antibody titres in univariate and multivariate analyses (multivariate ORs 3.453 [95% CI 1.019–11.710], 3.671 [95% CI 1.096–12.297] and 5.175 [95% CI 1.504–17.813], respectively), whereas gender, age, and vaccine dose were not (Table S6).

In Lambaréné, the highest dose (20 million pfu) was associated with strong Y4 ZEBOV-GP-specific antibody response in univariate and multivariate analyses (OR 9.289 [95% CI 1.013–85.149]), as

Table 2
Geometric mean titres of neutralizing antibodies by pseudovirus assay

A. USAMRIID laboratory

	Dose	*N* Day 0 GMT 95% CI	*N* Day 28 GMT 95% CI	*N* Month 6 GMT 95% CI	*N* Year 3 GMT 95% CI	*N* Year 4 GMT 95% CI	*N* Year 5 GMT 95% CI
Geneva	300 000 pfu	48 10.0 10.0–10.0	48 35.4 24.1–51.8	48 16.5 12.6–21.7	43 34.8 24.2–50.0	40 41.3 27.6–61.8	41 36.3 25.2–52.3
	10 million pfu	34 10.0 10.0–10.0	34 99.2 60.8–161.8	33 18.4 12.6–26.7	28 50.3 34.5–73.5	30 43.3 29.9–62.6	30 54.3 35.5–83.2
	50 million pfu	14 10.0 10.0–10.0	14 231.9 119.1–451.4	15 17.9 10.1–31.6	11 45.9 21.1–99.8	11 64.4 30.7–135.0	11 44.3 21.7–90.4
	10 or 50 million pfu	48 10.0 10.0–10.0	48 127.0 85.1–189.6	48 18.2 13.5–24.6	39 49.0 35.3–68.1	41 48.1 34.9–66.4	41 51.4 36.2–73.0
Lambaréné	300 000 pfu	19 10.0 10.0–10.0	19 42.9 19.0–96.8	16 11.6 8.4–16.1	17 31.3 17.7–55.4	17 18.2 11.0–30.0	ND
	3 million pfu	18 10.0 10.0–10.0	18 96.2 49.5–187.1	16 10.8 9.2–12.7	35 39.1 27.1–56.4	29 32.6 21.8–48.8	ND
	20 million pfu	ND	ND	ND	12 101.0 47.216.7	11 72.4 32.6–160.9	ND

B. Spiez laboratory

	Dose	*N* Day 0 GMT 95% CI	*N* Day 28 GMT 95% CI	*N* Month 6 GMT 95% CI	*N* Year 1 GMT 95% CI	*N* Year 2 GMT 95% CI	*N* Year 3 GMT 95% CI	*N* Year 4 GMT 95% CI	*N* Year 5 GMT 95% CI
Geneva	300 000 pfu	48 3.00 3.00–3.00	48 4.96 3.76–6.53	48 8.23 5.63–12.02	48 10.83 6.97–16.85	45 9.19 5.95–14.20	43 9.28 5.94–14.51	43 9.17 6.01–14.02	42 8.00 5.20–12.31
	10 million pfu	34 3.00 3.00–3.00	34 6.65 4.48–9.88	34 14.35 8.57–24.02	32 12.30 7.57–20.00	29 12.88 7.70–21.53	29 7.19 4.58–11.30	30 7.98 5.17–12.34	30 7.81 4.78–12.78
	50 million pfu	15 3.00 3.00–3.00	14 10.00 5.43–18.37	15 12.66 6.44–24.88	15 8.62 4.95–15.02	13 8.78 4.80–16.07	12 6.88 3.75–12.61	11 7.86 3.50–17.63	12 9.83 4.43–21.83
	10 or 50 million pfu	49 3.00 3.00–3.00	48 7.49 5.42–10.36	49 13.81 9.26–20.60	47 10.98 7.61–15.84	42 11.44 7.74–16.91	41 7.10 5.00–10.10	41 7.95 5.51–11.47	42 8.34 5.58–12.47

Panel A: USAMRIID laboratory, all vaccines. Panel B: Spiez laboratory, Geneva vaccines.
A PsV batch issue had previously resulted in artificially low PsV titres for the month 6 samples assessed at USAMRIID.
GMT, geometric mean titre; pfu, plaque-forming units; PsV, pseudovirus; USAMRIID, US Army Medical Research Institute for Infectious Diseases.

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were strong Y1 responses (OR 29.158 [95% CI 4.389–193.710]) and age <30 years (OR 6.379 [95% CI 1.038–39.165]; Table S6).

Discussion

VSV-ZEBOV-GP-binding antibodies are considered key mediators of early vaccine protection [17,18]. Their persistence is prolonged, as reflected by seropositivity in all volunteers at the last point assessed. We had previously reported a significant decline between their peak at D28–M3 and Y1 or Y2 (Geneva) after immunization [12]. Here we show that vaccine-induced antibodies plateau with no declining trend between Y2–Y4 (Lambaréné) or Y3–Y5 (Geneva). The twofold group titre increase between early and late samples is artefactual, resulting from their assessment in two batches separated by a SARS-CoV-2-related interruption. This increase was not observed in neutralizing-antibody titres. RCD curves confirm long-term ZEBOV-GP persistence in the entire population with, again, no declining trend during the final years of follow-up.

ZEBOV-GP-binding IgG avidity in Geneva vaccinees was as high as in EVD convalescents or the WHO reference standard. It was even higher in Lambaréné vaccinees, raising the question of post-immunization exposure to ZEBOV-GP cross-reacting antigens that would not affect GMTs, or of the influence of pre-immunization seropositivity, only present in Lambaréné vaccinees. A manuscript assessing avidity at each time point after immunization should soon shed light on this question.

Epitope targeting results suggest that a significant proportion of rVSV-ZEBOV-GP-induced antibodies are non-neutralizing, as only 20–30% targeted the base of GP1 and 20–60% the chalice bowl. Although a proportion of the KZ52-like GP1-targeting was observed in Lambaréné (20–50%), antibodies from EVD convalescents showed significantly higher blocking range (40–90%). If this difference is observed from M1 onwards in vaccinees, it could suggest distinct antigen recognition/shaping of the antibody repertoire following EVD and rVSV-ZEBOV immunization.

The use of two distinct PsV neutralization assays generated slightly different quantitative results, reflecting the numerous factors influencing such assays (origin of the glycoprotein for pseudotyping, number of viral particles to assess neutralization, varying read-out methods, etc.). Using wild-type EBOV to assess NA by SNT classically generates lower titres, as read-out relies solely on the assessment of fluorescence by microscopy. In addition, the larger size and the more complex, folded morphology of wild-type EBOV makes neutralization less straightforward and results in increased *in vitro* breakthrough infections when neutralization activity is low. The SNT thus resulted in undetectable NT already by Y1 in many samples. However, both PsV assays indicate the same trend: vaccinees remaining seropositive at Y1 saw no significant subsequent decline in NA. Altogether, this suggests that longer-term protective vaccine efficacy may rely both on low titres of NA and non-neutralizing ZEBOV-GP antibodies' exerting their function through other protective mechanisms, such as Fc-mediated properties [18].

As expected, strong early (D28 and mostly Y1) ZEBOV-GP-binding antibodies were the best predictors of sustained high antibody responses—with statistically significant influences in Geneva of vaccine-related arthritis, regardless of gender, age, or vaccine dose. In Lambaréné, the best predictors of antibody persistence were vaccine dose (20 million pfu) and young age.

This report has limitations, most notably the relatively small number of individuals by dose group who could be followed up to 4–5 years post-vaccination, and the influence of the SARS-CoV-2 pandemic on the processing of their samples at different times at USAMRIID.

These findings nonetheless have important public-health implications: Y1 titres, produced by long-lived plasma cells, may be a primary correlate of longer-term antibody persistence. Assessing how well antibodies persist 1 year post-vaccination may thus be a strong indicator of a vaccinee's ability to establish a pool of long-lived antibody-producing cells. These results provide no indication that a booster dose would be needed in the time frame studied. Additional studies will be needed, however, to confirm these findings and to extend both clinical and immunologic follow-up accordingly.

Author contributions

Writing—original draft: AH and C-AS; writing—review and editing: all authors; conceptualization: C-AS, AMH, DM, and TO; investigation: AH, STA, OE, JWH, SK, KR, TLC, HRJ, SSN, SR, PK, RZ, AMH, and C-AS; methodology: AH, OE, JWH, SR, AMH, and C-AS; formal analysis: AH, SK, KR, TLC, HRJ, SSN, SR, and RZ; supervision: OE, JWH, PK, AMH, and C-AS; project administration: AH and C-AS; and funding acquisition: DM and C-AS.

Transparency declaration

The authors declare no conflict of interest related to this work. In the last 3 years, Spiez Laboratory received funds from Molecular Partners, Roche, and the Bill and Melinda Gates Foundation for COVID-related research (OE, HRJ, SR, and RZ); AH received an institutional grant from LimmaTech Biologics for an epidemiological study on *Candida albicans*.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2023.08.026.

Appendix B. Consortia members

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UK: Sanjeev Krishna (St George's University of London).

Kenya: Philip Bejon, Patricia Njuguna (Kenya Medical Research Institute, Kilifi).

Switzerland: Claire-Anne Siegrist, Angela Huttner (Geneva University Hospitals, Geneva); and Marie-Paule Kieny, Vasee Moorthy, Patricia Fast, Barbara Savarese, Olivier Lapujade (World Health Organization, Geneva).

VSV-EBOVAC Consortium

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References

[1] Den Boon S, Marston BJ, Nyenswah TG, Jambai A, Barry M, Keita S, et al. Ebola virus infection associated with transmission from survivors. Emerg Infect Dis 2019;25:249–55. https://doi.org/10.3201/eid2502.181011.

[2] Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ca Suffit!). Lancet 2017;389:505–18. https://doi.org/10.1016/S0140-6736(16)32621-6.

[3] Mbala-Kingebeni P, Pratt C, Mutafali-Ruffin M, Pauthner MG, Bile F, Nkuba-Ndaye A, et al. Ebola virus transmission initiated by relapse of systemic Ebola virus disease. N Engl J Med 2021;384:1240–7. https://doi.org/10.1056/NEJMoa2024670.

[4] Malenfant JH, Joyce A, Choi MJ, Cossaboom CM, Whitesell AN, Harcourt BH, et al. Use of Ebola vaccine: expansion of recommendations of the advisory committee on immunization practices to include two additional populations – United States, 2021. MMWR Morb Mortal Wkly Rep 2022;71:290–2. https://doi.org/10.15585/mmwr.mm7108a2.

[5] Meeting of the strategic advisory group of experts on immunization, 22–24 march 2021: conclusions and recommendations. Weekly Epidemiological Rec (WER) 2021;96:197–216.

[6] Triplett CA, Niemuth NA, Cirimotich C, Meister G, Guebre-Xabier M, Patel N, et al. Immune correlates of protection from filovirus efficacy studies in non-human primates. Vaccines (Basel) 2022;10:1338. https://doi.org/10.3390/vaccines10081338.

[7] Grais R. Estimation of the correlates of protection of the rVSVΔG-ZEBOV-GP Zaire ebolavirus vaccine: a post-hoc analysis of data from phase 2/3 clinical trials. Lancet Microbe 2021;2.

[8] Gilchuk P, Murin CD, Cross RW, Ilinykh PA, Huang K, Kuzmina N, et al. Pan-ebolavirus protective therapy by two multifunctional human antibodies. Cell 2021;184:5593–5607.e18. https://doi.org/10.1016/j.cell.2021.09.035.

[9] Saphire EO, Schendel SL, Gunn BM, Milligan JC, Alter G. Antibody-mediated protection against Ebola virus. Nat Immunol 2018;19:1169–78. https://doi.org/10.1038/s41590-018-0233-9.

[10] Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, et al. Phase 1 trials of rVSV Ebola vaccine in Africa and europe. N Engl J Med 2016;374:1647–60. https://doi.org/10.1056/NEJMoa1502924.

[11] Regules JA, Beigel JH, Paolino KM, Voell J, Castellano AR, Hu Z, et al. A recombinant vesicular stomatitis virus Ebola vaccine. N Engl J Med 2017;376:330–41. https://doi.org/10.1056/NEJMoa1414216.

[12] Huttner A, Agnandji ST, Combescure C, Fernandes JF, Bache EB, Kabwende L, et al. Determinants of antibody persistence across doses and continents after single-dose rVSV-ZEBOV vaccination for Ebola virus disease: an observational cohort study. Lancet Infect Dis 2018;18:738–48. https://doi.org/10.1016/S1473-3099(18)30165-8.

[13] Huttner A, Dayer JA, Yerly S, Combescure C, Auderset F, Desmeules J, et al. The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: a randomised double-blind, placebo-controlled phase 1/2 trial. Lancet Infect Dis 2015;15:1156–66. https://doi.org/10.1016/S1473-3099(15)00154-1.

[14] Rudge Jr TL, Sankovich KA, Niemuth NA, Anderson MS, Badorrek CS, Skomrock ND, et al. Development, qualification, and validation of the Filovirus Animal Nonclinical Group anti-Ebola virus glycoprotein immunoglobulin G enzyme-linked immunosorbent assay for human serum samples. PLoS One 2019;14:e0215457. https://doi.org/10.1371/journal.pone.0215457.

[15] Zimmer G, Locher S, Berger Rentsch M, Halbherr SJ. Pseudotyping of vesicular stomatitis virus with the envelope glycoproteins of highly pathogenic avian influenza viruses. J Gen Virol 2014;95:1634–9. https://doi.org/10.1099/vir.0.065201-0.

[16] Torriani G, Trofimenko E, Mayor J, Fedeli C, Moreno H, Michel S, et al. Identification of clotrimazole derivatives as specific inhibitors of arenavirus fusion. J Virol 2019;93:e01744-18. https://doi.org/10.1128/JVI.01744-18.

[17] Medaglini D, Santoro F, Siegrist CA. Correlates of vaccine-induced protective immunity against Ebola virus disease. Semin Immunol 2018;39:65–72. https://doi.org/10.1016/j.smim.2018.07.003.

[18] Gunn BM, Lu R, Slein MD, Ilinykh PA, Huang K, Atyeo C, et al. A Fc engineering approach to define functional humoral correlates of immunity against Ebola virus. Immunity 2021;54:815–828.e5. https://doi.org/10.1016/j.immuni.2021.03.009.

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