Antibodies are necessary for rVSV/ZEBOV-GP–mediated protection against lethal Ebola virus challenge in nonhuman primates


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**Ebola viruses cause hemorrhagic disease in humans and nonhuman primates with high fatality rates. These viruses pose a significant health concern worldwide due to the lack of approved therapeutics and vaccines as well as their potential misuse as bioterrorism agents. Although not licensed for human use, recombinant vesicular stomatitis virus (rVSV) expressing the filovirus glycoprotein (GP) has been shown to protect macaques from Ebola virus and Marburg virus infections, both prophylactically and postexposure in a homologous challenge setting. However, the immune mechanisms of protection conferred by this vaccine platform remain poorly understood. In this study, we set out to investigate the role of humoral versus cellular immunity in rVSV vaccine-mediated protection against lethal Zaire ebolavirus (ZEBOV) challenge. Groups of cynomolgus macaques were depleted of CD4+ T, CD8+ T, or CD20+ B cells before and during vaccination with rVSV/ZEBOV-GP. Unfortunately, CD20-depleted animals generated a robust IgG response. Therefore, an additional group of vaccinated animals were depleted of CD4+ T cells during challenge. All animals were subsequently challenged with a lethal dose of ZEBOV. Animals depleted of CD8+ T cells survived, suggesting a minimal role for CD8+ T cells in vaccine-mediated protection. Depletion of CD4+ T cells during vaccination caused a complete loss of glycoprotein-specific antibodies and abrogated vaccine protection. In contrast, depletion of CD4+ T cells during challenge resulted in survival of the animals, indicating a minimal role for CD4+ T-cell immunity in rVSV-mediated protection. Our results suggest that antibodies play a critical role in rVSV-mediated protection against ZEBOV.**

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bola viruses (EBOVs) are enveloped, negative single-stranded RNA viruses with a genome of ~19 kb in size that belong to the Filoviridae family. There are five species of EBOV: Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SEBOV), Bundibugyo ebolavirus (BEBOV), Ébola virus (CIEBOV), and Reston ebolavirus (REBOV). The species vary in their pathogenicity, with ZEBOV being most pathogenic (up to 90% case fatality), followed by SEBOV and BEBOV, with up to 50%. CIEBOV and REBOV have been shown to be lethal in nonhuman primates (NHPs), but only CIEBOV has been associated with one severe human case so far (1, 2). Currently, Old World macaques, notably cynomolgus and rhesus macaques, are the gold standard animal model for studying ZEBOV pathogenesis and testing vaccines and therapeutics. Both macaque species are highly susceptible to ZEBOV, with development of viral hemorrhagic fever and 100% lethality (3).

Although there is no licensed vaccine or treatment available for EBOV infections, a number of vaccine platforms have proven to be efficacious in nonhuman primate challenge studies. These platforms include DNA, recombinant adenovirus (rAd) (alone or in combination with DNA prime), virus-like particles (VLPs), human parainfluenza virus 3, and recombinant vesicular stomatitis virus (rVSV) (4). Most of these vaccines express the ZEBOV glycoprotein (GP) as the immunogen. The rVSV approach has proven to be among the most promising vaccine platforms for ZEBOV. The rVSV vectors are based on a reverse genetics system for VSV serotype Indiana (5) and have also been used to develop immunization strategies against other viruses, like influenza virus (6) and simian/HIV (SHIV) (7). One dose of this vaccine can successfully protect rodents and nonhuman primates from lethal ZEBOV infection (8, 9). Additionally, a single dose of this vaccine confers partial protection postexposure in immunocompetent rodents and nonhuman primates as well as preexposure in immunocompromised SHIV-infected rhesus macaques against lethal ZEBOV challenge (10–12).

Little is known about the mechanisms of protection of the rVSV vectors against ZEBOV infection, although it appears that both cellular and humoral immune responses are required in the nonhuman primate infection model. In this study, we investigated the role of CD4+ T-cell, CD8+ T-cell, or CD20+ B-cell responses in conferring protection following vaccination with rVSV/ZEBOV-GP. To that end, we depleted these cell populations using monoclonal antibodies before and during the vaccination period with rVSV/ZEBOV-GP. Following depletions, we characterized the cellular and humoral response against ZEBOV-GP in vaccinated animals. Cellular responses were very low in all of the groups including the nondepleted animals. Interestingly, with the exception of the CD4+ T-cell–depleted group, all of the animals developed a ZEBOV-GP–specific IgG response. This included the CD20+ B-cell–depleted animals, suggesting that we were unable to completely eliminate the B cells in this group. More importantly, only the CD4-depleted animals succumbed to ZEBOV infection. To confirm that antibodies and not effector CD4+ T cells are critical for protection, additional animals were vaccinated and depleted of CD4+ T cells.
CD4+ T cells prior and during challenge with ZEBOV. These animals survived the infection, strengthening our conclusion that antibodies play a critical role in the protection mediated by the rVSV/ZEBOV-GP vaccine against lethal ZEBOV challenge.

**Results**

**Depletion Efficacy.** To identify the immune mechanisms of protection provided by the rVSV/ZEBOV-GP vaccine against lethal ZEBOV challenge, 20 cynomolgus macaques were divided into five groups: NHP1-4 rVSV/Marburg virus (MARV)-GP (negative control); NHP5-8 rVSV/ZEBOV-GP (positive control); NHP9-12 rVSV/ZEBOV-GP CD4+ T cell depleted; NHP13-16 rVSV/ZEBOV-GP CD8+ T cell depleted; and NHP17-20 rVSV/ZEBOV-GP CD20+ B cell depleted. One of the animals in the CD20 depletion group experienced an unexpected reaction to Rituximab during one depletion session and was humanely euthanized, leaving three animals in this group. T- and B-cell depletion regimens were initiated 7 d before vaccination (day v7; Fig. 1) to ensure that the targeted lymphocyte population was not present on the day of immunization. The frequency of CD4+ T cells, CD8+ T cells, and CD20+ B cells in peripheral blood mononuclear cell (PBMC) samples were monitored throughout the vaccination period by flow cytometry (FCM) (Fig. 2). The administration of anti-CD4 depleting antibody resulted in a significant reduction in the numbers of CD4+ T cells on the day of vaccination and a complete loss 7 d later (P < 0.001). The numbers of CD4+ T cells remained at nadir levels until 1 wk before challenge and likely beyond (Fig. 2A). Administration of CD8-depleting antibody resulted in complete loss of CD8+ T cells at the time of immunization in peripheral blood (Fig. 2B; P < 0.001). Numbers of CD8+ T cells began to slowly increase 7 d later, but even at day v21 the numbers had not reached prededepletion levels. Treatment with CD20-depleting antibody resulted in a complete loss of B cells at the time of vaccination in the peripheral blood (P < 0.001) lasting until day v21, at which point, a very small increase in numbers of B cells was detected in PBMCs (Fig. 2C). In summary, monoclonal antibody depletion was profound (achieving ~100% loss by day of vaccination) and lasted throughout the immunization period. Following depletion, recovery was very slow in all three lymphocyte subsets especially in the CD4+ T-cell compartment and the frequencies did not return to baseline by the time the animals were challenged with ZEBOV.

**T-Cell Proliferation Following Vaccination.** We next investigated the impact of T- and B-cell depletion on the development of vaccine-induced T-cell responses. Following antigen encounter, naïve T cells undergo a proliferative burst and differentiate into either central memory (CM) or effector memory T cells. The kinetics and magnitude of this proliferative burst can be assessed by measuring changes in the expression of Ki67, a nuclear protein associated with entry into the cell cycle using FCM. Our analysis revealed that proliferation within CD4+ and CD8+ T-cell subsets peaked on day v14 in all animals (Fig. 3). Interestingly, CD4 depletion did not result in a diminished CD8+ T-cell proliferation. On the contrary, the frequency of CD8+ CM T cells expressing Ki67 was higher in the CD4-depleted animals compared with nondepleted animals on days 14 and 21 after vaccination. This increase could potentially represent a compensatory mechanism. We also attempted to determine the frequency of ZEBOV-GP–specific T cells using IFN-γ capture enzyme-linked immunosorbent spot (ELISPOT), but in most animals, frequency of responding T cells was very low (Fig. S1A). This finding suggests that the bulk of the T-cell proliferation observed following vaccination is directed against VSV antigens rather than ZEBOV-GP.

**CD4+ T-Cell Depletion Results in Impaired B-Cell Responses.** We also evaluated B-cell proliferation and the development of ZEBOV-GP–specific IgG following vaccination. As described for T cells, B cells underwent a proliferative burst following vaccination that peaked at day v14 (Fig. 4A and B). As expected, B-cell proliferation was significantly compromised in CD4+ T-cell–depleted animals (P < 0.0001, 14 d after vaccination, and depletion effect, P < 0.05) (Fig. 4A and B). Moreover, CD4+ T-cell–depleted animals failed to generate a ZEBOV-GP–specific IgG response and their titers were indistinguishable from those of the negative control animals that received rVSV/MARV-GP and significantly lower than that of nondepleted rVSV/ZEBOV-GP–vaccinated animals (P < 0.0001, 14 d after vaccination; depletion effect, P < 0.01; Fig. 4C). These data highlight the importance of CD4+ T-cell help in the development of the humoral response. Nondepleted animals and CD8+ T-cell–depleted animals both generated a robust IgG response that peaked on day v21. Surprisingly, CD20-depleted animals generated an IgG response despite a profound depletion of B cells in peripheral blood (Fig. 2C). This IgG response was statistically indistinguishable from that generated by nondepleted or CD8+ T-cell–depleted animals. This observation suggests that CD20+ B-cell depletion in secondary lymphoid tissues such as draining lymph nodes and the spleen was most likely incomplete, resulting in the priming of an antibody response. To assess the neutralizing potential of the anti–ZEBOV-GP antibody response, we carried out a neutralizing antibody assay using serum from the day of ZEBOV challenge. Our results indicate that with the exception of the CD4+ T-cell–depleted group, all animals vaccinated with rVSV/ZEBOV-GP generated a robust neutralizing antibody response (Fig. 4D).

**CD4+ T-Cell-Depleted Animals Suffer from Infection.** Following vaccination, all animals were challenged with a lethal dose of ZEBOV [1 × 10⁷ focus-forming units (ffu)]. The hematological, clinical, and virological findings are summarized in Fig. 5 and Figs. S2 and S3. Animals that received rVSV/MARV-GP showed distinct signs of Ebola hemorrhagic fever (EHF) like pyrexia, anorexia, and maculopapular rash on face, chest, and limbs starting on day c4 requiring euthanasia 1 or 2 d later (Fig. 5A and B). The CD4+ T-cell–depleted animals showed similar signs of disease as the rVSV/MARV-GP–vaccinated animals and succumbed on days c7 and c8 (Fig. 5A and B). At the time of euthanasia, internal tissue damage characteristic for EHF including enlarged and necrotic liver and spleen was observed in both the rVSV/MARV-GP and the CD4-depleted animals (Fig. S2). ZEBOV titers were determined in the key tissues of EHF and confirmed the systemic spread (Fig. S3A). In contrast, all of the animals that developed a robust IgG response to ZEBOV-GP, including CD20+ B-cell–depleted animals, survived (Fig. 5A) and showed no signs of disease as illustrated by clinical scores that did not exceed those recorded at baseline (Fig. S5B).

In rVSV/MARV-GP and CD4+ T-cell–depleted groups, high levels of infectious virus were detected in the blood reaching upward of 10⁸ 50% tissue culture infectious dose (TCID₅₀/mL) blood on the day of euthanasia (Fig. 5C and Fig. S3B). However, none of the animals that survived ZEBOV challenge developed viremia with the exception of NHP18 (CD20 depleted), which showed a low level of transient viremia only by quantitative RT-PCR.

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**Fig. 1.** Study overview. Time line for vaccine, depleting antibody, and challenge virus administration as well as blood sample collection.
immune system to the fulminant viremia observed (Fig. S3) compared with c0. This increase in WBC is likely a response by the T cell depleted groups 4 d after challenge (Fig. S3). In addition to this thrombocytopenia, the white blood cell (WBC) count increased dramatically in the rSVSMARV-GP and CD4+ T cell depleted groups 4 d after challenge (P < 0.02 compared with c0). This increase in WBC is likely a response by the immune system to the fulminant viremia observed (Fig. S3). Interestingly, we also observed a statistically significant increase in WBCs in CD20+ B-cell–depleted animals 4 d after challenge (P < 0.01 compared with c0), which suggests a low-level viral replication in the tissues of these animals. Although the WBC count increased in rSVSMARV-GP and CD4+ T-cell–depleted animals, the number of lymphocytes in these animals dramatically decreased on day c4 (Fig. S3D; P < 0.01). This decrease could be due to EBOV-induced apoptosis of lymphocytes in the later stages of disease.

**ZEOBV-GP-Specific Antibodies Were Not Detected in Animals Succumbing to Infection.** Following challenge, we measured changes in ZEOBV-GP–specific IgG titers. Animals vaccinated with rSVSMARV-GP as well as the CD4+ T-cell–depleted animals did not have a detectable anti–ZEOBV-GP IgG response (Fig. 4C) on the day of challenge and succumbed to infection between days c5 and c8 (Fig. S4). In contrast, animals that received rSVZEOBV-GP and remained untreated or were CD8+ T cell or CD20+ B cell depleted generated a robust ZEOBV-GP–specific IgG response after vaccination (Fig. 4C). Following challenge, the ZEOBV-GP–specific IgG titer of animals in these three groups showed a significant increase (Fig. S4A). Interestingly, the CD8+ T-cell–depleted animals showed a higher increase in IgG titers than nondepleted controls 14 and 21 d after challenge (Fig. S4A). The increase in IgG titer was also accompanied by an increase in the neutralizing titer compared with prechallenge in all of the surviving animals (Fig. S4B).

We next determined IgG titers against the viral matrix protein ZEOBV-VP40 (Fig. S4C). Because this antigen was not a component of the vaccine, responses against it would indicate some ZEOBV replication after infection. Following ZEOBV challenge, all of the rSVZEOBV–vaccinated animals showed a modest increase in VP40-specific titers (Fig. S4C). One of the three CD20-depleted animals showed a more robust anti-VP40 response than the other animals (Fig. S4D). This was NHP18, the animal in which low levels of ZEOBV viremia were detected by qRT-PCR (Fig. S3B). These data show that the presence of antibodies protects against high levels of replication but may not provide sterile immunity and prevent infection.

**Cytokine Profiles After Challenge.** We examined the impact of T- and B-cell depletion on plasma levels of several cytokine and chemokines after challenge. Of the 14 soluble mediators that we examined, 6 showed changes associated with ZEOBV infection and with outcome. More specifically, IL-1β, IL-1ra, IL-10, IL-6, IFN-γ, and monocyte chemoattractant protein 1 (MCP-1) levels increased in both the rSVSMARV-GP–vaccinated and CD4+ T-cell–depleted animals shortly before these animals succumbed to infection (Fig. S5). Levels of MIP-1β also increased in the CD4-depleted animals shortly before euthanasia (Fig. S5). MIP-1α levels were below level of detection, and then increased dramatically on day 7 after challenge in CD4-depleted animals (Fig. S6). No changes in IL-17, IL-12/23p40 subunits, IL-15, IL-8, or TNFα with ZEOBV infection were detected (Fig. S6). These results suggest that lethal ZEOBV infection is associated with a cytokine storm with an increase in both proinflammatory and regulatory cytokines as previously described for rhesus macaques infected with ZEOBV strain Mayinga (13) as well as fatal human cases of EHF (14). Given the profound loss of lymphocytes in this challenge model (Fig. S3D), it is likely that endothelial cells lining the blood vessels are the main producers of these inflammatory cytokines leading to vascular permeability and leakage as reviewed elsewhere (15).

**CD4+ T Cells Play a Minimal Role in rSVSMediated Protection.** Our results thus far suggested a critical role for antibodies in the protection mediated by rSVSM against lethal ZEOBV challenge. However, because the CD20 depletion did not abrogate GP-specific IgG antibodies and only CD4-depleted animals succumbed to infection, the possibility that CD4+ T cells could play a role in
Although we observed a robust T-cell proliferative burst, the overall frequencies of ZEBOV-GP–specific T cells were low in all of the groups. This observation strongly suggests that the majority of the proliferating T cells were responding to rVSV antigens. In contrast, we detected a robust antibody response in all but the animals depleted of CD4+ T cells during vaccination and the group that received the rVSV/MARV-GP vaccine. The ability of CD20-depleted animals to generate ZEBOV-GP–specific antibodies most likely indicated a failure to deplete B cells in lymphoid organs. Following ZEBOV challenge, both the CD4+ T-cell–depleted and the rVSV/MARV-GP–vaccinated animals succumbed to disease. Animals within these two groups exhibited signs of EHF as indicated by decreased platelet counts, elevated liver enzyme levels, and a cytokine storm as previously described for humans (14). These results strongly suggest that failure to generate a ZEBOV-GP–specific IgG response results in lack of protection and fatal disease. However, the CD4-depleted animals were also lacking ZEBOV-GP–specific CD4+ T cells. To delineate the contribution of CD4+ T cells and antibodies to rVSV-mediated protection, we depleted an additional group of animals of CD4+ T cells shortly before and during challenge. These animals survived the lethal ZEBOV infection without exhibiting clinical symptoms of disease. These data demonstrate that CD4+ T cells do not play a critical role in rVSV-mediated protection against lethal ZEBOV infection, whereas the presence of ZEBOV-GP–specific antibodies was required for survival.

The data presented in this manuscript differ from earlier rodent studies that demonstrated that both CD8+ T cells and antibodies are required for protection from mouse-adapted ZEBOV (17). The potential role of CD8+ T cells in ZEBOV control is supported by clinical studies reporting that patients who died from EHF exhibited reduced numbers of activated CD8+ T cells compared with survivors (18). However, our data confirm and expand those reported by Jones et al. (19) where they determined the role of antibody and cytotoxic T-cell responses generated by the rVSV vaccine in mediating protection against mouse-adapted-ZEBOV challenge in mice. The authors performed T-cell depletion and serum transfer studies and showed that complete CD8+ T-cell depletion did not compromise protection, whereas passive transfer of CD4+ effector T cells versus antibodies, we added a fourth depletion group to our study. Four animals were vaccinated with rVSV/ZEBOV-GP and were allowed to generate normal T-cell and antibody responses (Fig. 6A). CD4+ T cells were then depleted 7 and 3 d before and 4 d after vaccination (Fig. 6B). These animals had unaltered CD8+ T cells and ZEBOV-GP–specific antibodies but no circulating CD4+ T cells at the time of challenge (Fig. 6B–D). Two additional control animals that received rVSV/MARV-GP and rVSV/ZEBOV-GP and remained nondepleted were included in this additional cohort. All animals were then challenged with 1,000 ffu of ZEBOV. As expected, the animal infected with rVSV/MARV-GP succumbed to infection 5 d after challenge (Fig. 6E). All of the CD4-depleted animals as well as the nondepleted control that received rVSV/ZEBOV-GP survived challenge (Fig. 6E). Animals depleted of CD4+ T cells before challenge did not experience disease symptoms (Fig. 6F) or the drop in platelet numbers associated with hemorrhagic disease (Fig. 6G). Interestingly, these animals failed to generate ZEBOV-VP40–specific antibodies most likely due to the lack of CD4+ T-cell help required for de novo B-cell responses during ZEBOV challenge (Fig. 6C). These data demonstrate that effector CD4+ T cells are not required for rVSV/ZEBOV-GP–mediated protection against lethal ZEBOV challenge.

Discussion

In the present study, we sought to identify the immune mechanisms of protection against lethal ZEBOV infection conferred by the rVSV vaccine platform expressing ZEBOV-GP as the viral immunogen in NHPs. Defining the immune correlates of protection against EBOV using clinical data has been difficult due to the high lethality and the sporadic nature of the outbreaks (16). In this study, we depleted cynomolgus macaques of CD4+, CD8+, and CD20+ lymphocytes by monoclonal antibody treatment before and during vaccination with the rVSV vectors. We then monitored the impact of these depletions on the development of the cellular and humoral responses against ZEBOV-GP, the viral immunogen.
Successful ZEBOV vaccine platforms in NHPs are consistently associated with antibody titers in survivors compared with nonsurvivors (20). These guinea pigs and cynomolgus macaques revealed statistically higher antibody titers after vaccination. Additionally, an analysis of vaccinated animal succumbed to ZEBOV infection, whereas animals depleted of CD4 T-cells prior and during challenge survived. (31). Platelet counts from EDTA blood sample collection. (D) Kinetics and magnitude of the T-cell–proliferative burst in response to rVSV vaccination before CD4+ T-cell depletion was determined using flow cytometry by measuring changes in the frequency of Ki67+ cells within CD4 and CD8 central and effecter memory subsets. (E) ZEBOV-GP– and ZEBOV-VP40–specific IgG titers were determined by ELISA on the day of and following challenge. (F) Frequency of circulating CD4+ T, CD8+ T, and CD20+ B cells was determined by flow cytometry. (G) Kaplan–Meier survival curves showing that the rVSV/MARV–GP–vaccinated animal succumbed to ZEBOV infection, whereas animals depleted of CD4+ T-cells prior and during challenge survived. (F) Average clinical scores during the challenge phase are shown. (G) Platelet counts from EDTA blood acquired throughout the study are depicted for each study group.

of immune serum 60 d after immunization resulted in 80% protection. Similarly, Geisbert et al. (12) showed rVSV/ZEBOV-GP vaccination failed to protect SHIV-infected macaques that failed to generate a ZEBOV-GP–specific IgG response following vaccination. More recently, a study by Wong et al. (20) showed that an adenovirus-based vaccine failed to protect mice genetically deficient in B or CD4+ T-cells, whereas mice lacking CD8+ T-cells were completely protected. Additional analyses of vaccinated guinea pigs and cynomolgus macaques revealed statistically higher antibody titers in survivors compared with nonsurvivors (20). These observations are in line with the results presented in this manuscript and strongly suggest that CD4+ T-cell help to B cells rather than CD4+ T-cell effector functions play a key role in protection. Taken together, these data point toward a critical role for antibodies in the protection conferred by the rVSV vaccine against ZEBOV.

This conclusion is in agreement with multiple reports that successful ZEBOV vaccine platforms in NHPs are consistently associated with the presence of antibodies (reviewed in ref. 21). Indeed, studies of NHP sera collected from animals vaccinated with various ZEBOV-GP–based vaccines identified that a prechallenge gp-specific titer of 3,700 was predictive of 100% survival (21). The critical role of antibodies in protection from EBOV is illustrated in clinical studies that examined humoral responses in EBOV-infected individuals and have reported that fatally infected individuals fail to generate an IgG or IgM response whereas survivors do (22). Moreover, during the 1995 outbreak in Kikwit, significant protection of ZEBOV-infected individuals was achieved by whole-blood transfusion from convalescent patients (23). Although in these studies the role of antibodies could not be distinguished from that of donor T cells and of hospital care that patients received, the data provide strong support for the role of antibodies in protection against fatal ZEBOV infection.

Earlier passive transfer studies in NHP models yielded conflicting results. Administration of hyper-IgG collected from horses hyperimmunized to ZEBOV delayed onset of disease but failed to protect NHPs against ZEBOV challenge (24). Similarly, the human monoclonal antibody KZ52, which displayed powerful neutralization in vitro and was able to protect guinea pigs in vivo (25), failed to protect rhesus macaques against lethal challenge with ZEBOV strain Kikwit when administered 24 h before challenge and again 4 d after challenge (26). Because only one epitope in ZEBOV-GP was targeted by this antibody, these data suggest the need to target multiple ZEBOV-GP epitopes during passive antibody treatment. This hypothesis is supported by data from four recent studies that have demonstrated a potent protective role for antibodies in postexposure prophylaxis. In the first study, polyclonal IgG were purified from several vaccinated NHPs that survived challenge with either ZEBOV or MARV. Passive transfer of this IgG as late as 48 h after virus challenge protected naïve NHPs against both MARV and ZEBOV lethal challenge (27). The second study in mice and guinea pigs used several ZEBOV-GP–specific monoclonal antibodies to target different epitopes and showed synergism between some pairs of monoclonal antibodies (28). In a more recent study in NHPs, a combination of two human–mouse chimeric neutralizing monoclonal antibodies were administered 1 d prior as well as 1 and 3 d after lethal ZEBOV challenge. This approach protected one out of three NHPs and prolonged time to death in a second animal (29). Finally, Qiu et al. (28) achieved full protection in NHPs using a postexposure treatment regimen with a mixture of three monoclonal antibodies when initiated within 24 h of ZEBOV infection. Initiation of the same treatment 48 h after lethal challenge resulted in 50% protection (30). Taken together, these results highlight the importance of targeting multiple epitopes in ZEBOV-GP especially in postexposure application.

Our data differ from those reported in a recent study using rAd5 as a vaccine vector that showed that CD8+ T-cell responses play a critical role for the protection against lethal ZEBOV infection in cynomolgus macaques (31). Furthermore, in this study, passive transfer of polyclonal IgG from rAd5-vaccinated animals failed to confer protection in naïve animals. In contrast, depletion of CD8+ T-cells in vaccinated animals before challenge abrogated protection (31). The difference in mechanisms of protection between rAd5 and rVSV could be mediated by differences in the type of vaccine-induced immune responses generated by these two vectors. Indeed, previous studies in a hamster model of Andes virus infection have shown that, whereas rAd5 engendered a robust cytotoxic T-cell response and a short-lived antibody response (32), the rVSV vaccine generated a potent neutralizing antibody response (33). Despite these differences, both vectors protected animals from lethal Andes virus challenge.

In summary, the data presented here indicate a critical role for antibodies in the protection against lethal ZEBOV infection after vaccination with rVSV/ZEBOV-GP and support the need for further studies on the role of antibodies as effective treatment for filovirus infections. Together with recent studies (27–29, 30, 31), there is indication that immune correlates of protection are likely
to be strongly influenced by the vaccine platform. These data suggest that cellular responses engendered by some vaccine platforms and humoral immunity induced by other vaccines can each provide protection. However, the unique characteristics of responses induced by different vaccination strategies that are important for protection remain to be uncovered. Future studies should compare the specificity and breadth of the B- and T-cell responses generated by leading vaccine platforms such as rAdV and rSVV. These studies will undoubtedly lead to the enhancement of current vaccine strategies.

Materials and Methods

Animal Ethics Statement. This study was approved by the Institutional Animal Care and Use Committee at the Oregon National Primate Research Center (ONPRC) and at the Rocky Mountain Laboratories (RML). Both ONPRC and RML are accredited by the American Association for Accreditation of Laboratory Animal Care (Public Health Service/Office of Laboratory Animal Welfare). The Animal Welfare Assurance numbers for both facilities are A3304-01 and A4149-01, respectively. SI Materials and Methods includes more information.

Vaccine Vectors and Challenge Virus. 1×10^9 plaque-forming units/mL of the ZEBOV-GP and rSVS-MARV-GP were administered on the day of vaccination (v0). 1×10^8 fuml/ml of ZEBOV strain Kikwit (passage 3) was used to challenge the animals. SI Materials and Methods includes more information.

Animal Studies and Sample Collection. A total of 26 male cynomolgus macaques (Macaca fascicularis), 4-6 y of age and S-11 kg in weight, were used in this study (n = 4/group). For detailed information, see SI Materials and Methods.

Viral Loads and Hematology. Viral loads were determined using qRT-PCR and titration assays as described previously (34). Blood cell count from whole blood was determined with the Hemavet 950FS Plus+ laser-based hematology analyzer (Drew Scientific). SI Materials and Methods includes more information.

Lymphocyte Proliferation and Humoral Immune Responses. PBMCs were surface stained with the indicated antibodies and the samples were analyzed using the LSRII instrument (Becton Dickinson) and FlowJo software (Tree Star). Antibody titers directed against ZEBOV-GP or ZEBOV-VP40 were measured by ELISA using plates coated with recombinant proteins. Neutralizing antibody titers were determined by performing focus reduction neutralization titration assays as described previously (34). For detailed information, see SI Materials and Methods.

ELISPOT Assay and Plasma Cytokine Levels. ELISPOT assay was carried out as previously described (35). Cytokine levels in postchallenge NHP sera were determined with Milliplex Non-Human Primate Magnetic Bead Panel as per manufacturer’s instructions (Millipore). SI Materials and Methods includes more information.

ACKNOWLEDGMENTS. We thank Julie Callison, Rachel LaCasse, and Kimberly Meade-White for excellent technical assistance. We are also grateful to Sandy Skogura, Jane Faris, Kathleen Meuschel, Amanda Weidlov, Edward Schreder-endrug, and Rocky Rivera for tremendous animal care. We also thank Monica Brown and Gina Nguyen for expert technical assistance. This work was funded by Pacific Northwest Regional Center for Excellence Grant U54 AI0811680, Oregon National Primate Research Center Core Grant P51 OD011092-53, and the Intramural Research Program, National Institute of Allergy and Infectious Diseases, National Institutes of Health.