

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.

Ebola 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), *Côte d'Ivoire ebolavirus* (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

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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 v-7; 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 predepletion 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 Succumb to Infection. Following vaccination, all animals were challenged with a lethal dose of ZEBOV [1×10^5 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. 5B).

In rVSV/MARV-GP and CD4⁺ T-cell-depleted groups, high levels of infectious virus were detected in the blood reaching upward of 10^8 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

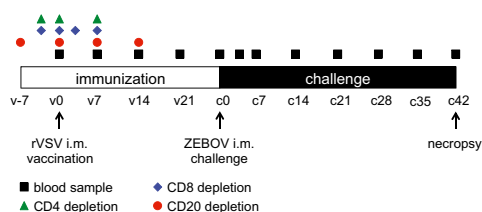


Fig. 1. Study overview. Time line for vaccine, depleting antibody, and challenge virus administration as well as blood sample collection.

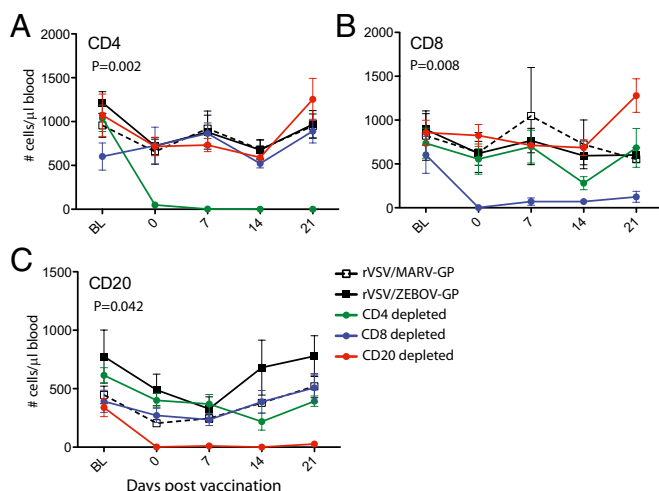


Fig. 2. Depletion efficacy. Circulating number of (A) CD4+ T, (B) CD8+ T, and (C) CD20+ B cells were determined by flow cytometry in rVSV/MARV-GP (open squares), rVSV/ZEBOV-GP (black squares), and rVSV/ZEBOV-GP that were CD4 (green), CD8 (blue), or CD20 (red) depleted. Depleting monoclonal antibodies resulted in profound depletion (*P* value for the overall depletion effect, <0.05) of the corresponding lymphocyte population by day 0, the time of vaccination. **P* < 0.05.

Fig. 3. Impact of lymphocyte depletion on T-cell proliferation following vaccination. Kinetics and magnitude of the T-cell proliferative burst in response to rVSV vaccination was determined using flow cytometry by measuring changes in the frequency of Ki67+ cells within CD4 (A and B) and CD8 (C and D) central and effector memory subsets. No data for CD4+ T-cell subsets in CD4-depleted animals and CD8+ T cells in CD8-depleted animals are provided because the frequency of these cells was too low to determine their proliferative status. Frequency of Ki67+ T cells peaked on day 14 in all subsets examined. Frequency of CD8 CM Ki67+ T cells was significantly higher in CD4+ T-cell-depleted animals on days 14 and 21 after vaccination: * $P < 0.05$.

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 rVSV/MARV-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.

ZEBOV-GP-Specific Antibodies Were Not Detected in Animals Succumbing to Infection. Following challenge, we measured changes in ZEBOV-GP-specific IgG titers. Animals vaccinated with rVSV/MARV-GP as well as the CD4+ T-cell-depleted animals did not have a detectable anti-ZEBOV-GP IgG response (Fig. 4C) on the day of challenge and succumbed to infection between days c5 and c8 (Fig. 5A). In contrast, animals that received rVSV/ZEBOV-GP and remained untreated or were CD8+ T cell or CD20+ B cell depleted generated a robust ZEBOV-GP-specific IgG response after vaccination (Fig. 4C). Following challenge, the ZEBOV-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 ZEBOV-VP40 (Fig. S4C). Because this antigen was not a component of the vaccine, responses against it would indicate some ZEBOV replication after infection. Following ZEBOV challenge, all of the rVSV/ZEBOV-GP-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 ZEBOV 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 ZEBOV infection and with outcome. More specifically, IL-1 β , IL-1ra, IL-10, IL-6, IFN- γ , and monocyte chemoattractant protein 1 (MCP-1) levels increased in both the rVSV/MARV-GP-vaccinated and CD4+ T-cell-depleted animals shortly before these animals succumbed to infection (Fig. S5). Levels of MIP-1 β also increased in the CD4-depleted animals shortly before euthanasia (Fig. S5). MIP-1 α 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 subunit, IL-15, IL-8, or TNF α with ZEBOV infection were detected (Fig. S6). These results suggest that lethal ZEBOV infection is associated with a cytokine storm with an increase in both proinflammatory and regulatory cytokines as previously described for rhesus macaques infected with ZEBOV 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 rVSV-Mediated Protection. Our results thus far suggested a critical role for antibodies in the protection mediated by rVSV against lethal ZEBOV 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

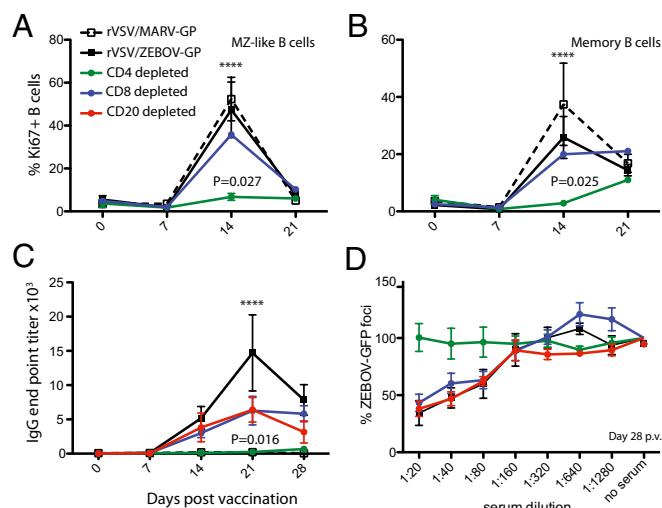


Fig. 4. Impact of lymphocyte depletion on humoral response following vaccination. Kinetics and magnitude of the B-cell proliferative burst was determined as described for T cells. (A and B) Frequency of Ki67+ MZ-like and memory B cells peaked 14 d after vaccination in all groups except for CD4+ T-cell-depleted animals that show minimal B-cell proliferation. B-cell frequencies were too low in the CD20+ B-cell-depleted animals to measure frequency of Ki67+ cells. (C) ZEBOV-GP-specific IgG endpoint titers were measured by ELISA. CD4+ T-cell-depleted animals generated a significantly reduced ZEBOV-GP-specific IgG response. (D) Neutralizing antibody titers for rVSV/ZEBOV-GP-vaccinated animals are shown. All groups with the exception of CD4+ T-cell-depleted animals generated a neutralizing antibody response. **** $P < 0.0001$.

protection remained. To delineate the relative contribution 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 challenge (Fig. 6A). 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.

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

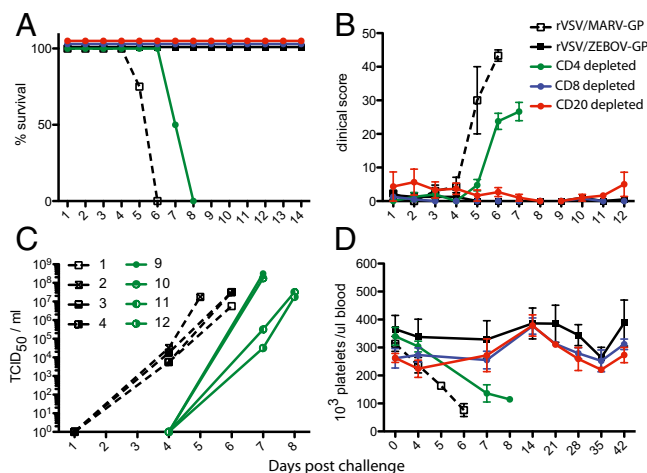
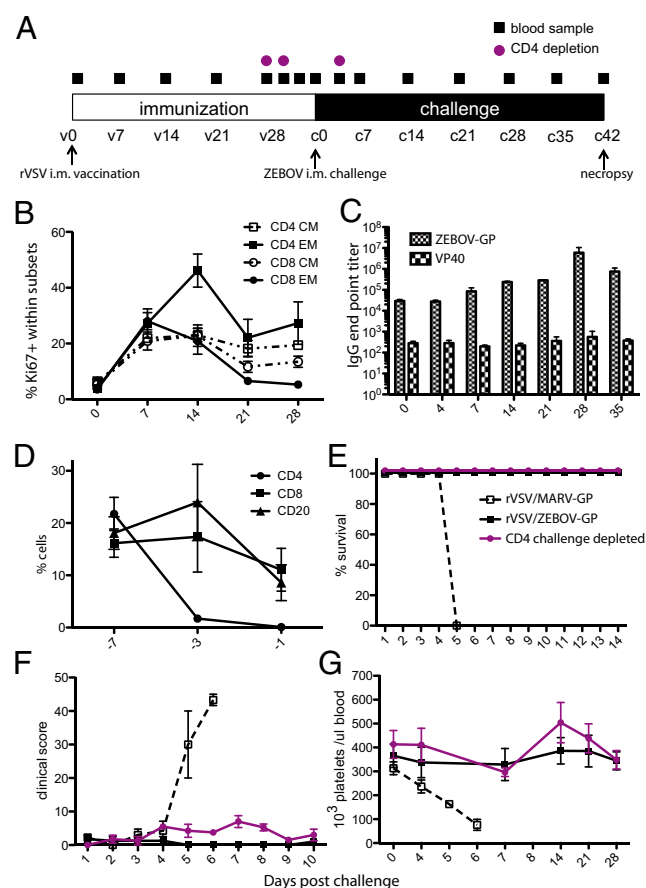


Fig. 5. Clinical data, survival, and viremia following ZEBOV challenge. (A) Kaplan–Meier survival curves showing that rVSV/MARV-GP-vaccinated and CD4+ T-cell-depleted animals succumbed to ZEBOV challenge. (B) Average clinical scores as obtained by an approved scoring sheet during the challenge phase are shown per study group. (C) Platelet counts from whole blood acquired throughout the study are depicted for each study group. (D) ZEBOV titers from whole-blood samples for each animal were determined on Vero E6 cells as 50% tissue culture infectious dose (TCID₅₀).



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 rAd5 and rVSV. 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 Animal Welfare Assurance numbers A3304-01 and A4149-01, respectively). *SI Materials and Methods* includes more information.

Vaccine Vectors and Challenge Virus. 1×10^7 plaque-forming units/mL rVSV/ZBOV-GP and rVSV/MARV-GP were administered on the day of vaccination (v0). 1×10^3 ffu/mL ZBOV 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 5–11 kg in weight, were used in this study ($n = 4/\text{group}$). 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 EDTA blood was determined with the HemaVet 950FS+ 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 ZBOV-GP or ZBOV-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.

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