Recombinant Vesicular Stomatitis Virus Vector Mediates Postexposure Protection against Sudan Ebola Hemorrhagic Fever in Nonhuman Primates

Thomas W. Geisbert, 1, 2, 3, 4* Kathleen M. Daddario-DiCaprio, 4, 5 Kinola J. N. Williams, 6
Joan B. Geisbert, 1 Anders Leung, 8 Friederike Feldmann, 8 Lisa E. Hensley, 5
Heinz Feldmann, 7, 8 and Steven M. Jones 6, 7, 8

National Emerging Infectious Diseases Laboratories Institute, 1 Department of Microbiology, 2 and Department of Medicine, 3 Boston University School of Medicine, 715 Albany Street, Boston, Massachusetts; Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, Maryland; 4 Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland; 5 Department of Immunology and Medical Microbiology, 7 University of Manitoba, 730 William Avenue, Winnipeg, Manitoba, Canada; and Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, 1015 Arlington Street, Winnipeg, Manitoba, Canada 8

Received 2 March 2008/Accepted 21 March 2008

Recombinant vesicular stomatitis virus (VSV) vectors expressing homologous filoviral glycoproteins can completely protect rhesus monkeys against Marburg virus when administered after exposure and can partially protect macaques after challenge with Zaire ebolavirus. Here, we administered a VSV vector expressing the Sudan ebolavirus (SEBOV) glycoprotein to four rhesus macaques shortly after exposure to SEBOV. All four animals survived SEBOV challenge, while a control animal that received a nonspecific vector developed fulminating SEBOV hemorrhagic fever and succumbed. This is the first demonstration of complete postexposure protection against an Ebola virus in nonhuman primates and provides further evidence that postexposure vaccination may have utility in treating exposures to filoviruses.

The filoviruses, Ebola virus (EBOV) and Marburg virus (MARV), cause severe and often fatal infections in humans and nonhuman primates. While there is a single species of MARV, there are four recognized species of EBOV: Ivory Coast ebolavirus (also known as Cote d’Ivoire ebolavirus), Reston ebolavirus, Sudan ebolavirus (SEBOV), and Zaire ebolavirus (ZEBOV) (7, 17). Until recently, nearly all EBOV outbreaks in humans have been caused by either SEBOV or ZEBOV. A possible fifth species of EBOV was associated with an outbreak in Uganda late in 2007, but little information is available regarding this new EBOV (1). Since 1976, there have been at least 10 outbreaks of ZEBOV, with case fatality rates approaching 90% (17). During the same period, there have been four outbreaks of SEBOV with mortality rates of approximately 50% (17).

Remarkable progress has been made over the last few years in developing candidate preventive vaccines that can protect nonhuman primates against EBOV and MARV (2, 3, 14, 15, 18–21). Advances in development of postexposure treatments and therapies against the filoviruses have been much slower. Some degree of success has been achieved using strategies that mitigate the coagulation abnormalities that characterize filoviral infection (10, 11, 13). Several new postexposure treatment approaches based on small interfering RNA (12) and antisense oligomers (6, 22) have shown promising results in rodent models, but there have been no published reports of either treatment strategy being evaluated in the more stringent macaque models. Recently, we showed the first complete postexposure protection of nonhuman primates against a filovirus by administering a live-attenuated recombinant vesicular stomatitis virus (rVSV) vector expressing the MARV glycoprotein (GP) shortly after a high-dose MARV challenge (4). We subsequently demonstrated that an rVSV vector expressing the ZEBOV GP protected 50% of rhesus macaques when admin-

* Corresponding author. Mailing address: Department of Microbiology, Boston University School of Medicine, 715 Albany Street, R514, Boston, MA 02118. Phone: (617) 638-4274. Fax: (617) 638-4286. E-mail: geisbert@bu.edu.

Published ahead of print on 2 April 2008.

FIG. 1. Kaplan-Meier survival curves for rhesus macaques given postexposure treatment for SEBOV infection.
TABLE 1. Clinical findings in rhesus monkeys infected with SEBOV and given postexposure treatment with an rVSV vector expressing the SEBOV GP or a VSV vector expressing a nonspecific GP

| Subject | Clinical finding(s) on:
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 6</td>
</tr>
<tr>
<td>1</td>
<td>Fever, lymphopenia,</td>
</tr>
<tr>
<td></td>
<td>thrombocytopenia, ALP†</td>
</tr>
<tr>
<td>2</td>
<td>Fever, lymphopenia</td>
</tr>
<tr>
<td>3</td>
<td>Fever, lymphopenia,</td>
</tr>
<tr>
<td></td>
<td>thrombocytopenia</td>
</tr>
<tr>
<td>4</td>
<td>Fever, lymphopenia, ALP†</td>
</tr>
<tr>
<td>Control 1</td>
<td>Fever, lymphopenia,</td>
</tr>
<tr>
<td></td>
<td>thrombocytopenia</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Subjects 1 to 4 underwent treatment with an rVSV vector expressing the SEBOV GP. Control 1 underwent treatment with a VSV vector expressing a nonspecific GP.

b Fever was defined as a temperature more than 2.5°F over baseline or at least 1.5°F over baseline and ≥103.5°F. Mild rash was defined as focal areas of petechiae covering less than 10% of the skin, moderate rash as areas of petechiae covering between 10% and 40% of the skin, and severe rash as areas of petechiae and/or ecchymosis covering more than 40% of the skin. Lymphopenia and thrombocytopenia were defined by a ≥35% drop in the numbers of lymphocytes and platelets, respectively. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; BUN, blood urea nitrogen; CRE, creatinine; UA, uric acid. †, two- to threefold increase; ††, four- to fivefold increase; †††, more-than-fivefold increase.

TABLE 2. Viral load in rhesus monkeys infected with SEBOV and given postexposure treatment with an rVSV vector expressing the SEBOV GP or a VSV vector expressing a nonspecific GP

| Subject | Plasma viral load (log_{10} PFU/ml) on:
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>1</td>
<td>0 (−)</td>
</tr>
<tr>
<td>2</td>
<td>0 (−)</td>
</tr>
<tr>
<td>3</td>
<td>0 (−)</td>
</tr>
<tr>
<td>4</td>
<td>0 (−)</td>
</tr>
<tr>
<td>Control 1</td>
<td>0 (−)</td>
</tr>
</tbody>
</table>

a Subjects 1 to 4 underwent treatment with an rVSV vector expressing the SEBOV GP, and control 1 underwent treatment with a VSV vector expressing a nonspecific GP.

b Viral load represents the log_{10} PFU of SEBOV/ml of plasma. Results in parentheses indicate whether the sample was positive (+) or negative (−) for SEBOV by RT-PCR. NT, not tested; NA, not applicable.
illness, and these animals remained healthy during the course of the study. One of these animals (subject 4) had a slight decrease in appetite on days 8 and 9, and another animal (subject 1) had a very small rash on one arm on days 9 and 10. Plaque assay was unable to detect any evidence of SEBOV in plasma of two of these three animals (Table 2); however, RT-PCR showed evidence of SEBOV in plasma of one of these animals at day 6 (subject 2) and the other animal at day 10 (subject 4). SEBOV was detected by plaque assay and RT-PCR in plasma of the third animal (subject 1) on days 6 and 10 (Table 2). The fourth animal treated with the VSVΔG/SEBOVGp vector (subject 3) developed symptoms consistent with SEBOV HF, including anorexia and a macular rash. SEBOV was detected by plaque assay and RT-PCR in plasma of this animal on days 6, 10, and 14 after challenge (Table 2). However, viral load never exceeded 4.2 log_{10} PFU/ml, a value which is thought to be an important indicator for predicting survival based on results of previous studies with filovirus-infected monkeys (T. W. Geisbert, unpublished observation). This animal cleared the viremia and showed little evidence of illness by day 20. In contrast, the control animal became severely ill and developed classic symptoms of SEBOV HF, including dehydration, anorexia, depression, and the presence of a macular rash. The control animal was the only animal that

FIG. 2. Serological response profile for rhesus macaques given postexposure treatment for SEBOV infection. (A) IgM. (B) IgG.
was febrile on days 10 and 14. SEBOV was detected in plasma on days 6, 10, 14, and 17 by plaque assay and RT-PCR, and the viral load exceeded 6.1 log_{10} PFU/ml by day 10 (Table 2).

The serological response profile of SEBOV infection after treatment was evaluated by IgM and IgG enzyme-linked immunosorbent assay. All four of the animals challenged with SEBOV and treated with the VSVΔG/SEBOVGP vector demonstrated moderate to high levels of IgM by day 6 (1:100 to 1:1,000) (Fig. 2A) and IgG by day 14 (1:1,000), while the control animal did not mount a humoral response against SEBOV (Fig. 2B).

Survival in this SEBOV study was better than the results of a similar study in rhesus macaques using the VSVΔG-ZEBOVGP vector as a treatment after a homologous ZEBOV challenge (8). In the ZEBOV study, we protected four of eight animals from lethal infection. The results of the current study were comparable to those of a study that we performed with rhesus macaques using the VSVΔG/MARVGP vector against homologous MARV challenge in which all specifically treated animals survived (4), although in the MARV study, none of the animals treated with the VSVΔG/MARVGP vector became viremic or clinically ill, whereas in the current study all of the animals treated with the VSVΔG/SEBOVGP vector became viremic as a result of the SEBOV challenge and one animal became clinically ill. The difference in outcomes among these three studies (SEBOV, ZEBOV, and MARV) may be due to the length of the disease course in rhesus macaques. That is, the window of opportunity may be larger for SEBOV and MARV than for ZEBOV. In this study with SEBOV, the control animal died on day 17. While very few studies have examined the pathogenesis of SEBOV HF in rhesus macaques, the disease observed in the control animals in this study is consistent with sparse historical data in which controls have succumbed 12 to 17 days after exposure (5; Geisbert, unpublished). Experimental infection of rhesus monkeys with MARV (Musoke strain) produces a uniformly lethal disease 11 to 13 days (mean, 11.6 days) after i.m. exposure to 1,000 PFU of MARV; experimental infection of rhesus monkeys with ZEBOV produces a uniformly lethal infection in 7 to 10 days (mean, 8.3 days) after i.m. exposure to 1,000 PFU of ZEBOV (11).

The exact mechanism of postexposure protection conferred by the VSVΔG/SEBOVGP vector remains uncertain. There have been no cases of survival or delay in death among the cohort of positive control macaques receiving rVSV vectors expressing nonspecific GPs (3, 4, 8, 15). Interference between the rVSVs and wild-type filoviruses cannot be discounted. All of the rVSV vectors expressing filoviral GPs replicate much faster in vitro than their counterpart wild-type filovirus (9; H. Feldmann, unpublished observation). As these rVSV vectors contain a full-length filoviral GP, they presumably target and infect the same preferred host cells as wild-type filoviruses (9). However, it is clear that an adaptive immune response is important for protection as we have seen previously that MARV-infected macaques treated with VSVΔG/ZEBOVGP died in parallel with untreated controls (4), showing that even though MARV and ZEBOV infect the same populations of host cells, an adaptive response is required to clear the virus and protect the host. Future studies to look at the biodistribution of rVSV in macaques and possible studies looking at depleting different immune cell populations may shed further light on this most interesting finding.

The current investigation was designed as a proof-of-concept study to compare the VSVΔG/SEBOVGP vector in the rVSV postexposure format with previous studies that used VSVΔG/MARVGP or VSVΔG/ZEBOVGP vectors. Clearly, studies are needed to look at how far out treatment can be delayed. For example, if MARV-infected or SEBOV-infected macaques are first treated with a homologous rVSV vector 24 h after challenge instead of 20 to 30 min, is complete protection still achieved? Regardless of outcome of this type of study; however, this strategy may still prove useful in treating potential laboratory accidents or medical personnel exposed during case patient management where rapid treatment is certainly feasible. In addition, the overall strategy of postexposure treatment using rVSV vectors expressing filoviral GPs may be even more useful against filoviruses that are associated with reduced mortality rates and where disease progresses more slowly, as may be the case with *Ivy Coast ebolavirus* (17) and/or with the apparent new EBOV species that was recently identified in Uganda (1).

From USAMRIID, we thank John Crampton and Carlton Rice for animal care and Anna Honko for assistance with biocontainment. From the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC), we thank Allen Grolla for technical assistance with biocontainment. We are grateful to John Rose (Yale University) for kindly providing us with the vesicular stomatitis virus reverse genetics system and Peter Jahrling (NIAID/NIH) for helpful discussions.

Work with filoviruses at USAMRIID was funded by the Defense Threat Reduction Agency (project number 04-4-J-0712). T.W.G. and J.B.G. were U.S. Government employees when portions of this work were performed at USAMRIID. Work with filoviruses at the NML was supported by PHAC. A grant awarded to H.F. by the Canadian Institutes of Health Research (MOP-39321), and a grant awarded to S.M.J. from Chemical, Biological, Radiological, and Nuclear (CBRN) Research and Technology Initiative (CRTL).

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by Boston University, the U.S. Army, or the Public Health Agency of Canada.

**REFERENCES**


