Stat1-Deficient Mice Are Not an Appropriate Model for Efficacy Testing of Recombinant Vesicular Stomatitis Virus–Based Filovirus Vaccines

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Stat1−/− mice lack a response to interferon α, β, and γ, allowing for replication of nonadapted wild-type (wt) Ebolavirus and Marburgvirus. We sought to establish a mouse model for efficacy testing of live attenuated recombinant vesicular stomatitis virus (rVSV)–based filovirus vaccine vectors using wt Ebolavirus and Marburgvirus challenge strains. While infection of immunocompetent mice with different rVSV-based filovirus vectors did not cause disease, infection of Stat1−/− mice with the same vectors resulted in systemic infection and lethal outcome for the majority of tested rVSVs. Despite differences in viral loads, organ tropism was remarkably similar between rVSV filovirus vaccine vectors and rVSVwt, with the exception of the brain. In conclusion, Stat1−/− mice are not an appropriate immunocompromised mouse model for efficacy testing of live attenuated, replication-competent rVSV vaccine vectors.

Keywords. Vesicular stomatitis virus; Ebolavirus; Marburgvirus; vaccine; Stat1-deficient mice.

Ebolavirus and Marburgvirus, members of the family Filoviridae, cause viral hemorrhagic fever in nonhuman primates and humans [1]. While outbreaks of filovirus hemorrhagic fever have occurred mainly in their endemic area in Central Africa, currently, the biggest documented Ebola virus (EBOV) outbreak is still ongoing in West African countries such as Guinea, Liberia and Sierra Leone. Not only has the virus spread from there into neighboring Mali, it was also introduced to Senegal, Nigeria, Spain, the United Kingdom, and the United States, where further spread of the virus was successfully stopped [2]. Despite tremendous efforts to accelerate phase 1 clinical trials for experimental vaccines and therapeutics (available at: http://www.ClinicalTrials.gov), there is still no approved countermeasure available. One of the experimental vaccine platforms currently in phase 1 clinical trials is based on recombinant vesicular stomatitis virus (rVSV). The rVSV vaccines for EBOV, which includes EBOV strain Mayinga (EBOV-May), and for Marburg virus (MARV), which includes MARV strain Musoke (MARV-Mus), are attenuated and have been shown to protect rodents and nonhuman primates from lethal disease [3], and vaccine vectors for other MARV isolates and ebolavirus species have since been developed [4].

Protective efficacy testing of vaccine candidates against challenge with filoviruses isolated directly from human or wildlife (wild-type [wt] isolates) can only be performed in macaques or immunocompromised rodents (ie, Stat1−/− or interferon α/β receptor [Ifnar]−/− mice), in which infection causes disease without previous adaptation [5, 6]. While Stat1−/− mice have been shown to develop lethal disease after infection with the majority of wt filovirus isolates [6], Ifnar−/− mice seem more resistant to infection with wt filoviruses (ie, Reston virus
[RESTV]) [5]. As the rVSV vector for EBOV-May did not cause disease in immunocompromised NOD-SCID mice [7], we sought to evaluate the usefulness of Stat1−/− mice as a model for the initial evaluation of the protective efficacy of new or improved rVSV filovirus vaccine vectors.

We hypothesized that the attenuated rVSV vectors would also be attenuated in this immunocompromised mouse model, in which rVSVwt causes lethal disease [8]. We found, however, that vaccination of Stat1−/− mice with 4 different monovalent rVSV vectors individually expressing different filovirus glycoproteins (GPs) resulted in systemic infection with lethal outcome for most of the rVSV vectors. In conclusion, while Stat1−/− mice are an animal model for wt Ebola virus and Marburg virus infections, this animal model can likely not be used for efficacy testing of live attenuated vaccines such as the rVSV-based vectors.

METHODS

Animal Ethics and Safety Statements
Research was approved and conducted in compliance with the guidelines of the National Institute of Allergy and Infectious Diseases/Rocky Mountain Laboratories Institutional Animal Care and Use Committee (IACUC). The facility where this research was conducted is fully accredited by the Office of Laboratory Animal Welfare Assurance (protocol A4149-01). All procedures were conducted by trained personnel under the supervision of veterinarians, and all invasive clinical procedures were performed while animals were anesthetized. Early end point criteria, as specified by the IACUC-approved scoring parameters, were used to determine when animals should be humanely euthanized. rVSV vectors are classified as biosafety level 2 (BSL-2) pathogens, and all work with the live vectors was approved by the Institutional Biosafety Committee (IBC) under BSL-2 conditions.

Virus Recovery, Propagation, and Purification
The following rVSVs were recovered from full-length plasmids and propagated as described previously [9–11]: rVSVwt, rVSV/EBOV-May-GP, rVSV/RESTV-Pen-GP (RESTV strain Pennsylvania), rVSV/MARV-Mus-GP, and rVSV/MARV-Ang-GP (MARV strain Angola). Viruses were subsequently purified through a 20% sucrose cushion, resuspended in Dulbecco’s modified Eagle’s medium (DMEM; Sigma, St. Louis, Missouri) containing 10% fetal bovine serum (FBS; Life Technologies, Carlsbad, California), aliquoted, and stored at −80°C. Viral titers were determined by performing classical plaque assay on Vero cells [11], and the median tissue culture infectious dose (TCID50) values were determined using the Reed–Muench method [12].

Growth Kinetics
Vero E6 cells were seeded in a 24-well plate the day before the experiment. Infection was performed with rVSVwt, rVSV/EBOV-May-GP, rVSV/RESTV-Pen-GP, rVSV/MARV-Mus-GP, and rVSV/MARV-Ang-GP at a multiplicity of infection of 0.001 for 1 hour. Following washing with plain DMEM, cells in each well were covered with 1 mL of DMEM/2% FBS. At 0, 6, 12, 24, 36, 48, 72, and 96 hours after infection, 30 µL of supernatant was collected. Samples were stored at −80°C before titration on Vero E6 cells.

Mouse Experiments
129S6/SvEv control and 129S6/SvEv-Stat1−/− mice aged 4–6 weeks (Harlan Laboratories) were inoculated with the indicated dose of rVSV (diluted in sterile DMEM) by intraperitoneal injection in a total volume of 200 µL. The animals were monitored for signs of illness and weighed daily. On necropsy days, mice were anesthetized, bled, and euthanized, and tissue samples were collected and stored at −80°C. Surviving mice were euthanized 21 days after infection, and serum specimens were collected for antibody titrations.

Titration
Vero E6 cells were seeded in a 96-well plate. Tissue samples were homogenized in DMEM and cleared of debris by centrifugation, and serial dilutions were prepared. Blood and cell culture supernatants were thawed, and serial dilutions were prepared. A confluent layer of Vero E6 cells was infected in triplicate per dilution for 1 hour at 37°C, and then 100 µL of DMEM/2% FBS was added. After 2–6 days, the plates were analyzed for VSV-specific cytopathic effect, and TCID50 values were calculated using the Reed–Muench method [12].

RESULTS AND DISCUSSION

For the development of filovirus vaccines, promising candidates will require efficacy testing, which involves challenging immunized animals with infectious wt Ebola virus and Marburg virus. In the case of small-rodent models, only the use of immunocompromised mouse strains or rodent-adapted viruses that cause severe disease are suitable for adequately evaluating vaccine efficacy. We sought to establish a mouse-screening model for efficacy testing of live attenuated vaccines, in which wt stocks of EBOV or MARV virions could be used to challenge immunized animals. We chose Stat1−/− mice, which lack a response to interferon α, β, and γ and have previously been shown to be largely susceptible to wt filovirus infections [6]. The potential feasibility of this approach was supported by the self-limiting replication of the first rVSV filovirus vector, rVSV/EBOV-May-GP, in immunocompromised NOD-SCID mice, resulting in an asymptomatic phenotype [7]. In contrast, VSVwt infection is known to cause lethal disease in Stat1−/− mice [8], but replication of rVSV
vectors expressing filovirus GPs instead of the VSV GP is known to be attenuated in vitro and in vivo [3, 10].

In a first experiment, we compared the growth kinetics of rVSVwt, rVSV/EBOV-May-GP, rVSV/RESTV-Pen-GP, rVSV/MARV-Ang-GP, and rVSV/MARV-Mus-GP in Vero E6 cells over 72 hours. As expected, 3 of the 4 different rVSV vectors replicated similarly in cell culture and were strongly attenuated (by several log10), compared with rVSVwt, but reached the same end titer at 72 hours. The exception was rVSV/MARV-Ang-GP, which was less attenuated over the same period (Figure 1A).

This finding is surprising, and further investigations need to address potential unique features of the MARV-Ang-GP that may influence infectivity, as the GP mediates virus entry and is the only difference among all vectors.

We next moved on to perform in vivo characterization of the rVSV vectors. First, groups of 3 129S6/SvEv control mice (immunocompetent) were intraperitoneally infected (the usual route of rVSV vaccination in mice) with 10^7 plaque-forming units (PFU)
of the 4 rVSV filovirus vectors and rVSVwt; none of the animals developed weight loss or other signs of disease (data not shown). Second, groups of 3 Stat1−/− mice were intraperitoneally infected with different doses (range, 10^4–10^7 PFU/animal) of the rVSV vectors and monitored daily for weight change and disease progression. As expected, rVSVwt caused lethal outcome in Stat1−/− mice at all doses tested (Figure 1B). Surprisingly, the only clearly attenuated vector was rVSV/MARV-Mus-GP, for which infection
resulted in 67%–100% survival (Figure 1C). While most of the mice survived, all infected animals displayed signs of disease, including weight loss (data not shown). Interestingly, rVSV/EBOV-May-GP was only slightly attenuated at the 2 lower doses, with all animals succumbing to infection in a dose-dependent manner (Figure 1D). Similarly, rVSV/RESTV-Pen-GP was only minimally attenuated in the groups receiving the two lower doses with increased time to euthanasia (Figure 1D). Interestingly, rVSV/MARV-Ang-GP was advanced in disease progression, with all animals succumbing to infection slightly faster than rVSVwt-infected mice, independent of the dose (Figure 1C).

All surviving immunocompetent control and Stat1−/− mice were euthanized on day 21 after infection, and serum samples were collected for the analysis of humoral immune responses. Every animal seroconverted to VSV, with titers of ≥102 400 confirming that all mice were infected with rVSV (data not shown). In addition, we compared GP-specific immunoglobulin G (IgG) responses in rVSV/MARV-Mus-GP infections of immunocompetent control and Stat1−/− mice, which developed IgG titers of up to 25 600 and 6400, respectively (data not shown). The lower titers detected in Stat1−/− mice were not unexpected, as these animals are defective in cell signaling pathways that contribute to T- and B-cell responses. In macaques, however, a MARV-Mus-GP-specific IgG titer of 640 has been sufficient for protection against lethal MARV-Mus challenge [3], [13], suggesting that the rVSV-immunized Stat1−/− mice might be protected from lethal filovirus challenge.

While it became obvious that Stat1−/− mice were not an appropriate model for testing the protective efficacy of rVSV filovirus vectors, we sought to characterize the disease caused by all the rVSV vectors in these mice. To determine the tissue tropism and organ virus load, we infected groups of 10 Stat1−/− mice with 102 PFU of the different rVSV vectors and euthanized 5 animals each 12 and 24 hours after inoculation. At the time of euthanasia, blood, lymph node, lung, heart, liver, spleen, kidney, and brain samples were collected and frozen for virus titration. As expected, the highest virus loads in all tissues were obtained for rVSVwt-infected animals, with titers comparable to previously published data from VSV infected Stat1−/− or Ifnar−/− mice [8], [14]. Notably, the lowest amounts of rVSVwt were consistently found in the brain of all mice (Figure 2A). Viremia and tissue titers were comparable for rVSVwt and rVSV/MARV-Ang-GP–infected mice at both time points (Figure 2A and 2B), confirming systemic infection and similar disease progression (Figure 1B and 1C). The only notable difference was found for brain tissue, in which rVSV/MARV-Ang-GP could only be detected in 1 of 5 mice at each time point (Figure 2B). Slightly lower virus titers were detected in blood and tissue samples from rVSV/EBOV-May-GP–infected mice (Figure 2C), mirroring the slightly slower disease progression, compared with rVSVwt-infected and rVSV/MARV-Ang-GP–infected animals (Figure 1B–D). As noted with rVSV/MARV-Ang-GP–infected animals, rVSV/EBOV-May-GP was only found to replicate in the brain of 1 mouse (Figure 2C). Infection of Stat1−/− mice with rVSV/RESTV-Pen-GP resulted in viremia similar to that in animals infected with rVSV/EBOV-May-GP, but virus tissue loads were lower and only detectable in liver, spleen, and lymph node (Figure 2C). This finding is in contrast to the lethal phenotype in Stat1−/− mice (Figure 1C) and warrants further investigation. rVSV/MARV-Mus-GP infection resulted in the lowest viremia level and in reduced viral tissue loads of all rVSV vectors (Figure 2B), which is in line with the attenuation of this vector (Figure 1D), but it still caused systemic infection without reaching the brain.

Notably, rVSV-infected immunocompetent control mice did not show any signs of disease, and virus was only occasionally isolated from lymph nodes, liver, or spleen samples from mice infected with any of the rVSV vectors, including rVSVwt. Overall, infection of immunocompetent control mice did not result in productive rVSV replication but was sufficient to generate humoral immune responses (discussed above). For rVSV/EBOV-May-GP, these antibody responses have previously been shown to protect mice from lethal mouse-adapted EBOV challenge [7].

While immunocompetent mice do not develop disease after infection with wt Ebola virus and Marburgvirus, it has been shown that Stat1−/− mice are susceptible to infection with these pathogens, leading to uniform lethality [5], [6]. Although the kinetics of disease progression differed among wt filoviruses, replication was similar for all, with key target organs being lymph nodes, liver, and spleen [6]. Overall, organ tropism of the rVSV filovirus vaccine vectors studied here in Stat1−/− mice was similar and confirms a key role of GP in determining cell and tissue tropism [1]. Interestingly, the organ tropism of rVSVwt was also similar [8], [14], which may be explained by the generally systemic nature of infections in Stat1−/− mice caused by wt filoviruses, rVSV filovirus vaccine vectors, and VSVwt. The only exception was the brain, which seems to be only a major target organ of VSVwt infection.

While Stat1−/− mice are not an appropriate animal model for evaluating the protective efficacy of live attenuated vectors such as the rVSV filovirus vaccines, this animal model might be useful for further studies characterizing the function of filovirus GPs. The remarkably different phenotypes displayed by the 2 rVSV/marburgvirus-GP and the 2 rVSV/ebolavirus-GP vectors in Stat1−/− mice are likely caused by different properties of their GPs, warranting further investigations.

Notes

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