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SUPPLEMENT ARTICLE

Ebola Virus Infection in Commonly Used Laboratory Mouse Strains

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The mouse model for Ebola virus (EBOV) is an established and often used animal model for countermeasure development. Although it has its limitations, it recapitulates certain key features of human EBOV disease and principally shows uniform lethality. However, in the recent past, several studies reported surviving animals when evaluating treatment or vaccine approaches. Therefore, we analyzed the severity of disease and lethality of mouse-adapted (MA-) EBOV infection in 6 different mouse strains. We identified outbred CD-1 mice to be the only strain tested resulting in uniform lethality when infected with different doses of MA-EBOV or reverse genetics–generated MA-EBOV. In contrast, infection of different inbred mouse strains resulted in partial survival depending on virus and dose. Of these inbred strains, 129 mice provided the most consistent model. Our study provides a helpful dataset when planning EBOV mouse studies for countermeasure efficacy testing and highlights the limitations of certain mouse strains as EBOV models.

Keywords. Ebola virus; mouse model; mouse-adapted; reverse genetics; BALB/c; C57BL/6; ICR (CD-1); 129.

Ebola virus (EBOV), the causative agent of the most recent West African epidemic, is a nonsegmented, single-stranded, negative-sense RNA virus in the family *Filoviridae*. At this time, there is no licensed treatment available against this deadly infection. However, many experimental treatment options have been assessed in animal studies, including monoclonal antibodies and small molecule inhibitors [1]. In addition, many vaccine approaches have been evaluated in preclinical studies against EBOV [2]. Almost every countermeasure candidate with promising in vitro efficacy is followed up by efficacy studies in mice, a well-established rodent model for EBOV infection. The mouse model was developed by Bray et al by serial passaging of EBOV in suckling mice, resulting in a uniformly lethal model in 3 mouse strains: BALB/c, CD-1, and C57BL/6 [3]. Although the model does not display all hallmark features of human EBOV disease, MA-EBOV infection causes pathologic changes, particularly in the liver and spleen, that closely resemble EBOV pathology in nonhuman primates [4]. However, recently, we and others observed that intraperitoneal MA-EBOV infection of BALB/c mice no longer results in uniform lethality. Especially when a treatment strategy is evaluated that requires daily intraperitoneal injection of a compound in solution, EBOV-infected BALB/c mice treated with control fluids such as phosphate-buffered saline have shown up to 30% survival (Haddock et al, unpublished data) [5]. Furthermore, EBOV infection of untreated BALB/c and C57BL/6 mice resulted in 19% and 9% survival, respectively [6, 7]. This is a concern particularly for C57BL/6 mice because many knockout mouse strains are based on this background and larger group sizes are needed to obtain statistically significant results.

In this study we compared the outcome of intraperitoneal MA-EBOV infection in different mouse strains (such as 3 different C57BL/6 strains—BALB/c, 129, and CD-1) commonly used in infectious disease research. Furthermore, we tested the influence of daily intraperitoneal treatment and age of the animals on outcome of infection. We found that all mouse strains were susceptible to infection resulting in severe and often lethal disease, but only CD-1 mice showed uniform lethality. Furthermore, lethal outcome of IP MA-EBOV infection in CD-1 mice was influenced by neither animal age nor daily intraperitoneal treatment.

METHODS

Animal Ethics and Biosafety Statement

All infectious work with MA-EBOV was performed in the maximum containment laboratory in accordance with standard operating procedures approved by the Rocky Mountain Laboratories Institutional Biosafety Committee, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health (Hamilton, Montana). All animal work was approved by the Institutional Animal Care and Use Committee (IACUC) and performed in strict accordance with the recommendations described in the Guide for the Care and Use of Laboratory Animals of the

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National Institutes of Health, the Office of Animal Welfare, and the United States Department of Agriculture in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. Food and water were available ad libitum. An IACUC-approved scoring sheet assisted in determination of the humane endpoint.

Viruses

Clonal, reverse genetics–derived rgMA-EBOV (passage 2) [8] and the original MA-EBOV (passage 3) [3] were propagated on Vero E6 cells, titered on these cells, and stored in liquid nitrogen. Virus dilutions for injection of 10 or 1000 focus-forming units (FFUs) per mouse were prepared immediately before challenge.

Mouse Strains

Female BALB/cAnNHsd (BALB/c), ICR (CD-1), and C57BL/6NHsd (C57BL/6N) mice were purchased from Envigo (Somerset, New Jersey). Female C57BL/6J and 129S1/SvlmJ (129) mice were purchased from the Jackson Laboratory (Bar Harbor, Maine). Female C57BL/6NCr were purchased from Charles River Laboratories (Wilmington, Massachusetts).

Challenge and Treatment

Mouse-adapted EBOVs were inoculated intraperitoneally by injecting 0.1 mL of suspension in Dulbecco's modified Eagle medium (DMEM) into 2 sites of the lower abdomen (0.2 mL total) as previously described [8]. Daily intraperitoneal placebo treatments of 0.1 mL of DMEM were injected into a single site 30–60 minutes after virus challenge and continued through day 10 after infection, alternating sides daily. All animals were monitored daily for body weight changes and at least once daily for clinical signs of disease. Animals were euthanized at >25% weight loss and/or signs of ataxia, extreme lethargy (unresponsive to touch), bloody discharge, tachypnea, dyspnea, or paralysis of limbs, as approved by the IACUC.

RESULTS

Lethality of Mouse-Adapted Ebola Virus Infection in 6 Different Laboratory Mouse Strains

The EBOV mouse model was established in the late 1990s by Bray and colleagues in adult female mice infected with an EBOV variant (MA-EBOV) obtained through serial passaging in suckling mice [3]. More recently, a clonal variant was generated by Ebihara and colleagues through reverse genetic techniques (rgMA-EBOV) that causes uniform lethality in BALB/c mice with a lethal dose causing 50% mortality (LD_{50}) of 0.01 FFUs [8]. Therefore, in this study we used MA-EBOV and rgMA-EBOV to infect 6-week-old female mice of the following strains—BALB/c, 129, CD-1, and C57BL/6. There are at least 3 C57BL/6 strains maintained commercially. In 1921, C. C. Little created the C57BL/6 mice, but by 1974 3 different strains , termed C57BL/6J (Jackson Laboratories), C57BL/6N (National Institutes of Health), and C57BL/6NCr (Charles River via National Institutes of Health), had been maintained [9]. These 3 strains can be distinguished genetically, with C57BL/6J mice displaying a deletion resulting in the absence of nicatinamide nucleotide transhydrogenase exons 7–11 that is not present in the other 2 strains [10]. We chose to use all 3 strains for our comparison.

Groups of 10 mice were infected via the intraperitoneal route with a low (10 FFUs/mouse) or high (1000 FFUs/mouse) dose of virus. The survival results for all groups are depicted in Table 1. All 6 strains of mice were susceptible to both MA-EBOV and rgMA-EBOV but displayed variable disease, ranging from mild to severe, with often lethal outcome demonstrated objectively by body weight loss (Supplementary Figure 1). Early clinical signs included ruffled fur and hunched posture, which were followed by lethargy and moribundity. Infection of the 3 C57BL/6 strains with either dose of rgMA-EBOV did not reveal uniform lethality (survival range: 20%–100%) (Table 1).

Table 1. Mouse Strain Survival After Infection With Mouse-Adapted Ebola Virus or Reverse Genetics–Derived Mouse-Adapted Ebola Virus

	Virus dose							
	MA-EBOV				rgMA-EBOV			
	10 FFU		1000 FFU		10 FFU		1000 FFU	
Mouse strain	Survival	Time to death, days after infection	Survival	Time to death, days after infection	Survival	Time to death, days after infection	Survival	Time to death, days after infection
C57BL/6N	44%[a]	7–9	10%	7–9	20%	7–15	90%	7
C57BL/6J	60%	7–9	90%	7	90%	8	100%	NA
C57BL/6NCr	30%	7–13	70%	7–9	60%	7–9	88%[a]	7
BALB/c	0%	3–7	10%	5–7	10%	4–7	40%	6–7
129	0%	5–7	10%	6–8	0%	6–7	0%	6–8
CD-1	0%	5–7	0%	5–6	0%	5–7	0%	5–6

Abbreviations: FFU, focus-forming unit; rgMA-EBOV, reverse genetics–derived mouse-adapted Ebola virus; MA-EBOV, mouse-adapted Ebola virus; NA, not applicable

[a]n = 9 in these 2 groups only; all others: n = 10.

Interestingly, increased survival was observed when mice were infected with the high dose (1000 FFUs). Similarly, C57BL/6J and C57BL/6NCr mice injected with high-dose MA-EBOV also showed increased survivalwhen compared with mice infected with low-dose MA-EBOV; however, this was not the case for C57BL/6N mice (Table 1). C57BL/6NCr mice lethally infected with rgMA-EBOV died 7–9 days after infection, similar to C57BL/6N and C57BL/6J mice infected with MA-EBOV. However, the C57BL/6NCr mice infected with the low dose showed an extended time to death of 7–13 days after infection. In contrast with the relatively high survival rates of all C57BL/6 mice (10%–100% survival), BALB/c mice showed low survival (0%–10%) when infected with MA-EBOV; this rate increased to 40% survival when they were infected with high-dose rgMA-EBOV (Table 1). BALB/c mice succumbed to intraperitoneal infection faster (3–7 days after infection), showing consistently earlier weight loss than C57BL/6 or 129 mice (Supplementary Figure 1). The 129 mice displayed the lowest survival rates of all tested inbred strains when infected with 10 FFUs (no survival) or 1000 FFUs (10% survival) of MA-EBOV and uniform lethality when infected with rgMA-EBOV at both doses. It is interesting to note that, in general, high-dose infection resulted in increased survival in most inbred mouse strains used in this study, except for 129 mice. The outbred CD-1 mice were the only mouse strain that showed uniform lethality independent of virus and dose. These mice succumb quickly, with only a few clinical signs preceding moribundity. Both 129 inbred and CD-1 outbred mice have not only the lowest survival rates but also the narrowest window in time to death at 5–7 days after infection (Table 1).

Lethal Dose Causing 50% Mortality of Mouse-Adapted Ebola Virus in CD-1 Mice

Because only CD-1 mice showed uniform lethality following MA-EBOV and rgMA-EBOV infection at 6 weeks of age, we completed a dose-finding study with MA-EBOV in these mice infected via the intraperitoneal route to determine the LD_{50} (n = 6 per dose; dose range, 0.001–10 000 FFUs). Because of evidence that daily placebo intraperitoneal injections could impact survival, we performed testing with and without daily intraperitoneal injection of 0.1 mL of DMEM starting 30–60 minutes after infection for 10 days. Mice were monitored throughout for body weight and clinical signs. We determined the LD_{50} of MA-EBOV in CD-1 mice to be 0.01 FFUs, with full lethality achieved at a dose as low as 1 FFU (Table 2). Surprisingly, daily DMEM injection increased disease progression and lethality following MA-EBOV infection with a LD_{50} of 0.005 FFUs and full lethality at 0.01 FFUs. The range in time to death following MA-EBOV infection without DMEM injection at any dose was 5–9 days after infection, with the animals succumbing earlier as the dose increased. Following infection with daily DMEM injection, the range in time to death was widened (5–11 days after infection), but narrowed as the dose increased (Table 2).

Table 2. Lethal Dose Causing 50% Mortality Determination of Mouse-Adapted Ebola Virus Infection in CD-1 Mice

	CD-1 survival (n = 6)			
	Daily treatment			
	–		+	
Virus dose	Survival	Time to death, days after infection	Survival	Time to death, days after infection
0.001 FFU	100%	NA	100%	NA
0.01 FFU	50%	6–8	0%	7–13
0.1 FFU	16.7%	6–7	0%	6–11
1.0 FFU	0%	6–8	0%	5–9
10 FFU	0%	6–7	0%	6–8
100 FFU	0%	6–7	0%	5–10
1000 FFU	0%	5–7	0%	5–8
10 000 FFU	0%	5–6	0%	5–6
	LD_{50} = 0.01 FFU		LD_{50} = 0.005 FFU	

Groups of 6 mice were intraperitoneally infected with mouse-adapted Ebola virus at the noted dose, with or without additional daily intraperitoneal injections of Dulbecco's modified Eagle medium.

Abbreviations: FFU, focus-forming unit; LD_{50}, lethal dose causing 50% mortality; NA, not applicable

Influence of Age on Mouse-Adapted Ebola Virus Infection in CD-1 Mice

The most common use of the EBOV mouse model is efficacy testing of treatment compounds or vaccines. CD-1 mice at 6 weeks of age appear ideal for the former; however, vaccination protocols incur additional aging time in mice that could reduce susceptibility to MA-EBOV infection. We therefore tested infection kinetics in CD-1 mice at ages ranging from 6 weeks to 14 weeks, assuming a prime-boost vaccination protocol with 4-week intervals. Groups of mice (n = 6) were infected via the intraperitoneal route with 1000 LD_{50} (10 FFUs) MA-EBOV and monitored for clinical signs and body weight changes. This dose was uniformly lethal at all ages (Table 3), and the age made no significant difference in either weight loss or time to death (Supplementary Figure 2C). In parallel, we infected mice at the

Table 3. Influence of Mouse Age on the Outcome of Mouse-Adapted Ebola Virus Infection

	CD-1 survival (n = 6)			
	Daily treatment			
	–		+	
Mouse age	Survival	Time to death, days after infection	Survival	Time to death, days after infection
6 wk	0%	5–7	0%	6–7
8 wk	0%	5–7	0%	5–7
10 wk	0%	6–7	0%	6–8
12 wk	0%	5–8	0%	5–7
14 wk	0%	6–7	0%	6–7

Groups of 6 mice of the noted ages were intraperitoneally infected with 10 focus-forming units of mouse-adapted Ebola virus (1000 lethal dose causing 50% mortality), with or without additional daily intraperitoneal injections of Dulbecco's modified Eagle medium.

same age distribution with 1000 LD_{50} MA-EBOV followed by daily intraperitoneal injections of 0.1 mL of DMEM. Again, lethality was uniform despite treatment (Table 3) or age, and treatment made no significant difference in weight loss or time to death of the mice (Supplementary Figure 2*D*).

DISCUSSION

This study analyzed disease progression and outcome of MA-EBOV infection in 6 different mouse strains. Surprisingly, the 2 inbred mouse strains originally reported to show uniform lethality following MA-EBOV infection—BALB/c and C57BL/6 [3]—showed different degrees of survival to infection with MA-EBOV or rgMA-EBOV. Interestingly, for BALB/c and C57BL/6 mice, infection with the higher virus dose resulted in increased survival, a phenomenon that has been reported previously [5]. The reason for this observation remains unknown. Conceivably, increased amounts of defective-interfering particles associated with high-dose infection might delay productive EBOV infection and thus influence disease outcome, as has been described for other viruses [11]. Possibly, higher incoming virus load could lead to faster and stronger stimulation of innate responses [12] that are normally effectively dampened by EBOV through viral-encoded interferon antagonism [13]. This does not explain why this phenotype was not observed in 129 or CD-1 mice. Thus, future studies need to clarify this interesting phenomenon.

Inbred mice are often preferred over outbred strains to minimize variations in study outcome and overall reduce animal numbers. Thus, based on this study, 129 mice seem to be an obvious choice as a lethal EBOV inbred mouse model. However, more recent studies targeted at elucidating pathogenic mechanisms of EBOV infection use a variety of knockout mice often derived from C57BL/6 mice. C57BL/6 mice are also favored for certain haplotype-restricted immunology studies. Regrettably, the H-2^b C57BL/6 model demonstrated much higher survival following MA-EBOV and rgMA-EBOV infection, and disease outcome varied greatly dependent on the different C57BL/6 mouse strains analyzed. Although the BALB/c model was also not uniformly lethal, infection with MA-EBOV resulted in a lower level of survival, making it an alternative H-2^d option for such research purposes. Again, perhaps the best alternative for H-2^b immunologic work might be the 129 mice.

Countermeasure work benefits from uniform lethality in animal models because statistically significant results can be achieved with reduced animal numbers. If not restricted to inbred mouse strains, the use of CD-1 mice as a lethal EBOV mouse model seems the obvious choice because these mice present with the appearance of defined clinical signs, uniform lethality, and a tight window for time to death. Additionally, the model is not affected by daily intraperitoneal injections, and a wide age range seems similarly susceptible to lethal MA-EBOV infection. Moreover, CD-1 mice are quite docile, of larger size, and substantially less expensive than the inbred strains tested in this study, which reduces costs as well as difficulties in handling and blood sampling in maximum containment. On the downside, CD-1 mice may be less optimal for certain immunology studies because they are an outbred strain with greater genetic variability and thus more likely to differ in host responses to infection. However, this can also be advantageous because they may provide a stronger predictive value than inbred mouse strains when moving countermeasure testing into larger outbred mammalian species such as guinea pigs or nonhuman primates on the path to licensure. All together, this research favors CD-1 mice as a valuable EBOV mouse model for efficacy studies on antivirals, therapeutics, and vaccines.

In conclusion, we characterized the value of 6 different laboratory mouse strains as EBOV mouse models. Only outbred CD-1 mice provided uniform lethality, with 129 mice being the most consistent model among the tested inbred strains. This information will help in better planning of future EBOV countermeasure efficacy studies.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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References

1. Cross RW, Mire CE, Feldmann H, Geisbert TW. Post-exposure treatments for Ebola and Marburg virus infections. Nat Rev Drug Discov **2018**; [Epub ahead of print] doi: 10.1038/nrd.2017.251.
2. Reynolds P, Marzi A. Ebola and Marburg virus vaccines. Virus Genes **2017**; 53:501–15.
3. Bray M, Davis K, Geisbert T, Schmaljohn C, Huggins J. A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. J Infect Dis **1999**; 179(suppl 1):S248–58.
4. Bradfute SB, Warfield KL, Bray M. Mouse models for filovirus infections. Viruses **2012**; 4:1477–508.
5. Bounds CE, Kwilas SA, Kuehne AI, et al. Human polyclonal antibodies produced through DNA vaccination of transchromosomal cattle provide mice with post-exposure protection against lethal Zaire and Sudan Ebolaviruses. PLoS One **2015**; 10:e0137786.
6. Marzi A, Ebihara H, Callison J, et al. Vesicular stomatitis virus-based Ebola vaccines with improved cross-protective efficacy. J Infect Dis **2011**; 204(suppl 3):S1066–74.
7. Marzi A, Reinheckel T, Feldmann H. Cathepsin B & L are not required for Ebola virus replication. PLoS Negl Trop Dis **2012**; 6:e1923.

8. Ebihara H, Takada A, Kobasa D, et al. Molecular determinants of Ebola virus virulence in mice. PLoS Pathog **2006**; 2:e73.
9. Song HK, Hwang DY. Use of C57BL/6N mice on the variety of immunological researches. Lab Anim Res **2017**; 33:119–23.
10. Toye AA, Lippiat JD, Proks P, et al. A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice. Diabetologia **2005**; 48:675–86.
11. Roux L, Simon AE, Holland JJ. Effects of defective interfering viruses on virus replication and pathogenesis in vitro and in vivo. Adv Virus Res **1991**; 40:181–211.
12. Caballero IS, Honko AN, Gire SK, et al. In vivo Ebola virus infection leads to a strong innate response in circulating immune cells. BMC Genomics **2016**; 17:707.
13. Messaoudi I, Amarasinghe GK, Basler CF. Filovirus pathogenesis and immune evasion: insights from Ebola virus and Marburg virus. Nat Rev Microbiol **2015**; 13:663–76.