Lethality and pathogenesis of airborne infection with filoviruses in A129 α/β $-/-$ interferon receptor-deficient mice

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Normal immunocompetent mice are not susceptible to non-adapted filoviruses. There are therefore two strategies available to establish a murine model of filovirus infection: adaptation of the virus to the host or the use of genetically modified mice that are susceptible to the virus. A number of knockout (KO) strains of mice with defects in either their adaptive or innate immunity are susceptible to non-adapted filoviruses. In this study, A129 α/β $-/-$ interferon receptor-deficient KO mice, strain A129 IFN- α/β $-/-$, were used to determine the lethality of a range of filoviruses, including Lake Victoria marburgvirus (MARV), Zaire ebolavirus (ZEBOV), Sudan ebolavirus (SEBOV), Reston ebolavirus (REBOV) and Côte d'Ivoire ebolavirus (CIEBOV), administered by using intraperitoneal (IP) or aerosol routes of infection. One hundred percent mortality was observed in all groups of KO mice that were administered with a range of challenge doses of MARV and ZEBOV by either IP or aerosol routes. Mean time to death for both routes was dose-dependent and ranged from 5.4 to 7.4 days in the IP injection challenge, and from 10.2 to 13 days in the aerosol challenge. The lethal dose (50 % tissue culture infective dose, TCID₅₀) of ZEBOV for KO mice was <1 TCID₅₀ ml⁻¹ when administered by either the IP or aerosol route of infection; for MARV the lethal dose was <1 TCID₅₀ ml⁻¹ by the IP route of infection and <10 TCID₅₀ ml⁻¹ by the aerosol route. In contrast, there was no mortality after infection with SEBOV or REBOV by either IP or aerosol routes of infection; all the mice lost weight (~15 % loss of group mean body weight with SEBOV and ~7 % with REBOV) but recovered to their original weights by day 14 post-challenge. There was no mortality in mice administered with CIEBOV via the IP route of infection and no clinical signs of infection were observed. The progression of disease was faster following infection with ZEBOV than with MARV but ultimately both viruses caused widespread infection with high titres of the infectious viruses in multiple organs. Histopathological observations were consistent with other animal models and showed widespread organ damage. This study suggests that MARV and ZEBOV are more virulent when administered via the IP route rather than by aerosol infection, although both are highly virulent by either route. The KO mouse may provide a useful model to test potential antiviral therapeutics against wild-type filoviruses.

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INTRODUCTION

The family *Filoviridae* currently consists of two genera, *Ebolavirus* and *Marburgvirus*. The genus *Ebolavirus* includes

Côte d'Ivoire ebolavirus (CIEBOV, also known as Tai Forest ebolavirus), Reston ebolavirus (REBOV), Sudan ebolavirus (SEBOV), Zaire ebolavirus (ZEBOV) and Bundibugyo ebolavirus (BEBOV). The genus *Marburgvirus* currently contains only one recognized species, Lake Victoria marburgvirus (MARV) (Kuhn, 2008). Of the above viruses, MARV, SEBOV, BEBOV and ZEBOV infections cause severe viral haemorrhagic fever with a high case-fatality rate in humans (Sanchez *et al.*, 2007; CDC, 2009) and have low infection doses for non-human primates (Groseth *et al.*,

Abbreviations: ACDP, Advisory Committee on Dangerous Pathogens; BEBOV, Bundibugyo ebolavirus; CIEBOV, Côte d'Ivoire ebolavirus; CL4, containment level 4; H&E, haematoxylin and eosin; IP, intraperitoneal; KO, knockout; MARV, Lake Victoria marburgvirus; REBOV, Reston ebolavirus; SEBOV, Sudan ebolavirus; TCID₅₀, 50 % tissue culture infective dose; ZEBOV, Zaire ebolavirus.

2007). CIEBOV has been associated with only one, non-lethal case in a human but is lethal for non-human primates. REBOV is considered avirulent for humans but seroconversion (and therefore infection, presumably) has been described (Barrette *et al.*, 2009; WHO, 2009). In addition, REBOV, SEBOV and BEBOV are lethal with reduced virulence for non-human primates (Bray, 2002).

Filoviruses are Hazard Group 4 (HG4) agents and must, therefore, be handled within a UK Advisory Committee on Dangerous Pathogens (ACDP) containment level 4 (CL4) laboratory (equivalent to a BSL-4). They are listed as 'category A biothreat agents' by the US Centers for Disease Control (CDC, 2009). Filoviruses may be transmitted to laboratory animals experimentally by the airborne route (Johnson *et al.*, 1995; Lub *et al.*, 1995a, b; Bazhutin *et al.*, 1992). Indeed, airborne transmission may have occurred during an outbreak of REBOV infection in a primate housing facility (Jaax *et al.*, 1995). More recently, humans have seroconverted following potential aerosol exposure in pig farms in the Philippines (Barrette *et al.*, 2009). The filoviruses appear stable in small-particle aerosols (Piercy *et al.*, 2010) and may be expected to prove most hazardous if disseminated by this route.

Several animal species have been used to model filovirus infection; however, non-human primates are generally believed to be the model most representative of human disease (Bray & Paragas, 2002). Normal immunocompetent mice are not susceptible to infection with naturally occurring filoviruses (Bray, 2001), hence there are two strategies available to establish a murine model: either adaptation of the virus to the host or the use of genetically modified susceptible mice.

Adaptation of the viruses to infect wild-type mice has been achieved for both MARV and ZEBOV (Bray *et al.*, 1998; Warfield *et al.*, 2009). However, virus adaptation leads to genetic changes that make extrapolation of the behaviour of wild-type virus unreliable and may provoke concerns with licensing authorities.

A number of gene knockout (KO) mouse strains with defects in either their adaptive or innate immunity are susceptible to wild-type filoviruses (Bray, 2001). Here, we examine the infectivity, lethality and pathogenesis of filoviruses in strain A129 IFN- α/β $-/-$ receptor-deficient mice, by administering doses of virus via intraperitoneal (IP) injection and, for the first time, via aerosol routes of infection. The filoviruses chosen were (strains are given in parentheses): wild-type MARV (Popp), ZEBOV (E718), SEBOV (Boniface), CIEBOV (1994 isolate) and REBOV (Pennsylvania). To our knowledge, neither of the strains of MARV or ZEBOV chosen here has previously been studied in this model.

METHODS

Viral growth. All manipulations of viruses were performed within an ACDP CL4 laboratory at the Defence Science and Technology

Laboratory (Dstl), Porton Down. The viruses were kindly supplied by Dr G. Lloyd, HPA Porton Down, UK. Human-derived ZEBOV strain E718 (Vero cell passage 3), SEBOV strain Boniface (Vero cell passage 3), REBOV (Vero cell passage 8), CIEBOV (Vero cell passage 4) and MARV strain Popp (Vero cell passage 7) were cultured in Vero C1008 cells (ECCAC cat. no. 85020206) in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with Sigma medium constituents 2% FCS, 1% HEPES, 3 mmol L-glutamine l^{-1} and 100 units each penicillin and streptomycin ml^{-1} . Virus-infected cell cultures were incubated at 37 °C in a 5% CO₂ humidified atmosphere for 7 days prior to harvesting viruses. Infected tissue culture supernatant was clarified by centrifugation at 900 g for 15 min, followed by titration and storage at -80 °C.

Virus enumeration assay. Viral titres were estimated by end-point dilution as described previously (Piercy *et al.*, 2010). Briefly, 100 μ l virus sample was added to each well of the first column of a 96-well tissue culture plate containing 100% confluent monolayers of Vero C1008 cells. Serial tenfold dilutions of virus (final volume 180 μ l) were prepared across eight rows of the plate up to column 9, column 10 was left blank and columns 11 and 12 contained Vero cells alone as negative controls. Plates were incubated at 37 °C in a 5% CO₂ humidified atmosphere for 6 days before the addition of 3 μ l (~1.5%) neutral red dye (Sigma) and further incubation overnight. Cell monolayers were fixed for 30 min by the addition of 100 μ l 10% formal saline per well followed by the removal of excess fluid. Cytopathic effects, characterized by cell rounding and monolayer destruction, were determined and used to identify virus-infected cells and neutral red staining was used to visualize viable, metabolically active Vero cells. The 50% tissue culture infectious dose (TCID₅₀) was derived using the formula of Reed & Muench (1938).

Animal experiments. Adult male and female strain A129 mice aged 6–9 weeks lacking the IFN- α/β receptor (IFN- α/β $-/-$) and normal parental strain A129 mice (IFN- α/β $+/+$) were used (B&K Universal). Animal studies were performed in accordance with the UK Scientific Procedures Act (Animals) 1986 and UK Codes of Practice for the Housing and Care of Animals Used in Scientific Procedures, 1989. Infected animals were housed in rigid-walled isolators, under negative pressure and with *ad libitum* access to food and water, in a dedicated ACDP CL4 animal laboratory. Mice were observed twice daily for clinical signs or mortality. All animals were acclimatized for 1 week in the isolators before entering an experiment.

Lethality studies. Groups of five mice were inoculated with various doses of filovirus, administered by either IP injection (0.1 ml) or exposure to aerosol, and observed for clinical signs. Weights of individual mice were recorded daily. Infectious aerosols were generated using a Collison nebulizer containing filovirus suspensions at various concentrations. Airborne particles produced in this manner are predominantly 1–3 μ m in diameter (Thomas *et al.*, 2008). The infectious aerosol was conditioned in a modified Henderson apparatus (Henderson, 1952) and the mice, in nose-only exposure chambers, were exposed for 10 min to a dynamic aerosol at 50–55% relative humidity and 22 ± 3 °C, by using standard techniques (Druett, 1969; Lever *et al.*, 2009). Briefly, the dose of filovirus to which each mouse was exposed was derived from the titration of virus present in impinger samples, collected into DMEM (supplemented as above) during infection (May & Harper, 1957). The dose of infectious virus received by each mouse was derived from the formula: $(a \times b \times c \times d) / e \times (f/1000)$, where a =impinger count (TCID₅₀ ml^{-1}), b =impinger volume (ml), c =minute volume of animal ($ml\ min^{-1}$), d =exposure time (min), e =impinger flow rate ($l\ min^{-1}$) and f =impinger sample time (min). The respiratory minute volume of a mouse was taken as 20 $ml\ min^{-1}$ (Guyton, 1947) and the retained dose was estimated based upon the assumption that each mouse retained

40 % of the inhaled dose (Harper & Morton, 1962). Impinger flow rates were 12 l min⁻¹.

Pathogenesis study. Groups of mice were exposed to MARV or ZEBOV by using the aerosol route of transmission as described above. The challenge, with both viruses, was with a medium dose that resulted in a retained TCID₅₀ of 10 per mouse. At various time points post-challenge, between 0 and 9 days for MARV or 3 and 8 days for ZEBOV, two to three mice were culled and organs were assayed for virus infection as described above.

At the same time points, tissue samples from liver, spleen, kidney, lung and brain were fixed in 10 % buffered formalin solution (Sigma) and processed for paraffin wax embedding using standard techniques for histopathological analysis. Sections ~5 µm thick were prepared and stained with haematoxylin and eosin (H&E).

RESULTS

Lethality following challenge via IP injection

Groups of A129 IFN- α/β $-/-$ mice were challenged with a range of doses of different filoviruses administered via the IP route (Table 1). All the A129 IFN- α/β $-/-$ mice that were challenged with various doses of either ZEBOV or MARV by this route succumbed at 4–5 and 5–10 days post-challenge, respectively (Table 1, Fig. 1). There was a dose-dependent response regarding time to death, which was reflected in the intensity of the clinical signs (data not shown). There was no mortality in mice challenged with various IP doses of

SEBOV or CIEBOV, or with a single high dose (10⁵ TCID₅₀) of REBOV. Clinical signs, including piloerection and lethargy, preceded death by 1–2 days in mice challenged with ZEBOV and MARV. Mice challenged with IP doses of SEBOV or REBOV became anorexic, leading to ~15 % and ~7 % loss of group mean body weight, respectively, by day 7 post-challenge (Fig. 1). These animals later recovered and regained a group mean body weight indistinguishable from that of the non-infected animals. There was no significant weight loss in mice challenged with IP doses of CIEBOV (Fig. 1).

Lethality following aerosol challenge

Groups of A129 IFN- α/β $-/-$ and parental wild-type IFN- α/β $+/+$ mice were challenged with different calculated retained doses of ZEBOV and MARV or a single dose of SEBOV administered via the aerosol route. There was no sign of clinical symptoms, weight loss or mortality in control wild-type (IFN- α/β $+/+$) A129 mice challenged by the aerosol route with ZEBOV, MARV or SEBOV (results not shown). All the A129-IFN α/β $-/-$ mice challenged with various aerosol doses of ZEBOV and MARV succumbed at between 8 and 13 days post-challenge (Table 1, Fig. 1). There was a dose-dependent response regarding mean time to death in mice challenged with MARV; however, there was no such response with the ZEBOV challenge. Clinical signs in ZEBOV- and MARV-infected mice included lethargy,

Table 1. Lethality of filoviruses following IP and aerosol routes of infection in A129 IFN- α/β $-/-$ mice

ND, Not determined.

Virus strain	IP route			Aerosol route		
	Challenge dose (log ₁₀ TCID ₅₀ ml ⁻¹)	Mean time to death (days)	Mortality rate (%)	Retained dose (log ₁₀ TCID ₅₀ ml ⁻¹)	Mean time to death (days)	Mortality rate (%)
MARV	5.8	5.40	100	2.92	11.0	100
	4.8	5.00	100	2.62	10.2	100
	3.8	7.00	100	1.92	12.0	100
	2.8	7.20	100	0.92	13.0	100
	1.8	7.40	100			
	0.8	8.75	80			
	-0.2	8.75	80			
	-1.2	10.0	40			
ZEBOV	2.0	4.00	100	2.00	8.0	100
	1.0	5.75	100	1.00	8.0	100
	0	5.20	100	0	8.0	100
SEBOV	4.8	—	0	1.11	—	0
	3.8	—	0			
	2.8	—	0			
	1.8	—	0			
REBOV	5.0	—	0	ND	ND	ND
CIEBOV	5.0	—	0	ND	ND	ND
	4.0	—	0			
	3.0	—	0			
	2.0	—	0

Fig. 1. Change in weight over time of A129 IFN- α/β $-/-$ mice infected with MARV and some ebolaviruses by the IP and aerosol route. Groups of A129 IFN- α/β $-/-$ mice were challenged with different filoviruses, administered either by IP (a) or aerosol (b) routes, and weighed daily. The mean percentage weight change is shown. (a) Mice were challenged with approximately $10 \text{ TCID}_{50} \text{ ml}^{-1}$ ZEBOV ($\bullet$), $63.2 \text{ TCID}_{50} \text{ ml}^{-1}$ MARV ($\square$), $63.2 \text{ TCID}_{50} \text{ ml}^{-1}$ SEBOV ($\blacktriangledown$), $1.0 \times 10^5 \text{ TCID}_{50} \text{ ml}^{-1}$ REBOV ($\blacklozenge$) and $1.0 \times 10^5 \text{ TCID}_{50} \text{ ml}^{-1}$ CIEBOV (∇). Non-infected wild-type mice ($\times$) were used as a control. (b) Mice were challenged via aerosol route with a calculated retained dose of approximately $10 \text{ TCID}_{50} \text{ ml}^{-1}$ ZEBOV ($\bullet$), $8.4 \text{ TCID}_{50} \text{ ml}^{-1}$ MARV ($\square$) and $13 \text{ TCID}_{50} \text{ ml}^{-1}$ SEBOV ($\blacktriangledown$). Non-infected wild-type mice ($\times$) were used as a control.

weight loss and piloerection and began 24–48 h prior to death.

Mice challenged with SEBOV exhibited anorexia from day 7 to 11 post-challenge (~30% reduction in group mean body weight) (Fig. 1); however, by ~24 days post-challenge, all SEBOV-infected mice had recovered, returning to the weight of the non-infected controls. REBOV and CIEBOV were not tested by the aerosol route as they did not cause mortality when administered by the IP route.

Pathogenesis following aerosol challenge

Viral load increased in all organs over time (Fig. 2), with ZEBOV being recovered earlier post-challenge than MARV. Both viruses were recovered from lungs at all time points, with the highest titres obtained from blood. MARV could only be recovered from the kidney and brain during the terminal stages of infection (day 9) but was present earlier in other organs. In contrast, ZEBOV was recovered from all organs tested, with peak virus levels occurring between days 6 and 8 (consistent with the onset of clinical signs).

Histopathology following aerosol challenge

Histopathological lesions were observed in H&E-stained sections of lung, liver and kidney from two-thirds of mice at day 3 post-ZEBOV challenge (Fig. 3). From day 6

post-challenge, histopathological lesions in the liver, lung, spleen and kidney were consistently present in all the mice examined. Congestion and multifocal necrosis, apoptotic hepatocytes (day 7 onwards) and megalocytosis (day 8 post-challenge) were consistent features in liver tissue samples (Fig. 3f). Lymphoid follicle depletion, numerous apoptotic bodies and tingible-body macrophages were present in spleen samples and congestion with inflammatory infiltrates were seen in the kidneys (Fig. 3b, d). In lung tissue samples, congestion occurred in all animals examined on day 3 post-challenge. Perivascular oedema was present in two-thirds of mice at day 6 post-challenge with haemorrhage and hypertrophy of bronchiolar epithelium observed in one mouse. By day 7 post-challenge, pulmonary congestion was still evident, as was perivascular and peribronchiolar oedema, which were also seen in mice on day 8 post-challenge and after death.

Hypertrophy of bronchiolar epithelium of the lung was evident at day 2 post-MARV challenge in all animals tested. Other pathological features were similar to those seen following ZEBOV infection, with signs of liver (Fig. 4), lung, spleen and kidney tissue damage by day 5 post-challenge and further indications of tissue damage visible at days 7 and 9 post-MARV challenge. A single mouse had meningeal congestion in the brain on day 7 post-MARV infection, which was the only pathological feature of the brain observed (not shown).

Fig. 2. Viral titres over time in A129 IFN- α/β $-/-$ mice infected with MARV or ZEBOV by the aerosol route. Viral titres of ZEBOV (●) or MARV (□) were measured in A129 IFN- α/β $-/-$ mice at various times post-infection with a retained dose of 10 TCID₅₀ ml⁻¹ of either virus. Counts in the lung, liver, spleen, blood, kidney and brain were determined by the TCID₅₀ assay. Error bars represent SEM.

DISCUSSION

Non-human primates are widely believed to be the best representative animal model for human filovirus disease (Bente *et al.*, 2009), displaying clinical and pathological features similar to those seen in humans. Normal immunocompetent mice are not susceptible to infection with non-adapted filoviruses, and guinea pigs develop a mild to moderate febrile disease (with ZEBOV and SEBOV), with associated anorexia and weight loss, then recover (Bray & Paragas, 2002).

The complexities of working with non-human primates, particularly under maximum BSL-4 containment conditions, mean that smaller animal models for filovirus disease would be more beneficial for screening potential therapeutics, studying pathogenesis and performing lethality studies prior to testing in primates. Smaller animal models may also be more easily adapted for potency assays during the development and licensure of promising therapeutics.

To this end, studies have been conducted using a mouse-adapted virus, ZEBOV-76 (Bray *et al.*, 1998), which was uniformly lethal for immunocompetent BALB/c mice infected via the IP route but non-lethal when delivered via aerosol. More recently, a mouse-adapted MARV, derived by sequential passage in SCID mice, was found

to be lethal when delivered via IP injection (Warfield *et al.*, 2007, 2009). Licensure of any filovirus therapeutics or vaccines, however, is likely to require testing with fully virulent isolates derived from human diseases of known passage history.

The A129 IFN- α/β $-/-$ mouse strain was highly susceptible to a range of filoviruses, including ZEBOV-76, SEBOV strain Boniface, MARV strain Musoke and the guinea pig-adapted MARV strains Musoke and Ravn (Bray, 2001). These mice were also susceptible to mouse-adapted ZEBOV (although ZEBOV isolated from the 1995 outbreak in Zaire proved non-lethal). It has been suggested that a gradient of filovirus virulence exists, demonstrable by infection of a range of normal and immunodeficient mouse strains, of which the ZEBOV 1976 outbreak isolate and SEBOV strain Boniface appear to be the most virulent, followed by guinea pig-adapted MARV strain Ravn and guinea pig-adapted and non-adapted MARV strain Musoke (both REBOV and CIEBOV caused no illness) (Bray, 2001). This gradient appears to reflect the virulence and associated mortality seen in human infections with the various filovirus strains available.

The present study compared lethality in A129 IFN- α/β $-/-$ mice infected via either the IP or aerosol route of transmission with wild-type strains of MARV and four

Fig. 3. Histopathology of organ tissues from A129 IFN- α/β $-/-$ mice at different time points post-infection with ZEBOV via the aerosol route. Tissues were stained using H&E stain. A129 IFN- α/β $-/-$ mice were exposed to a retained dose of $10 \text{ TCID}_{50} \text{ ml}^{-1}$ aerosolized ZEBOV. (a) Lung tissue at day 3 post-challenge showing mild hypertrophy of the bronchiolar epithelium (arrow). (b) Spleen tissue at day 6 post-challenge showing marked lymphoid depletion of the lymphoid follicles. (c) Lung tissue at day 6 post-challenge showing perivascular haemorrhages (arrows) and mild hypertrophy of the epithelium (arrowhead). (d) Spleen tissue at day 7 post-challenge showing marked lymphoid depletion and the presence of multiple apoptotic bodies and tingible-body macrophages (arrows). (e) Lung tissue at day 7 post-challenge showing perivascular oedema (arrow). (f) Liver tissue at day 7 post-challenge showing multifocal necrotic hepatitis (arrows). Non-infected control tissues for comparison are provided in Fig. 4(c–e).

species of EBOV. To our knowledge, the aerosol route had not been investigated previously and our data proved consistent with a gradient of virulence, where ZEBOV strain E718 was the most virulent followed by MARV (Popp) with REBOV and CIEBOV being the least virulent. Although deficient in innate immunity, the A129 IFN- α/β $-/-$ mice clearly retained the ability to resist filovirus infection as mice were also protected by vaccination (unpublished data). In contrast to the findings of Bray (2001), in our studies, SEBOV (strain Boniface) caused disease but was non-lethal. Such attenuation of SEBOV was unexpected and may be a consequence of sequential passage of this virus in humans (Bowen *et al.*, 1980) as infections seen during the later stages of the epidemic were less severe (WHO, 1978) and the strain of SEBOV used was isolated after an estimated 10–11 passages in humans.

Laboratory passage in Vero cells or animals may also have altered the virulence of this virus (Bowen *et al.*, 1980; Bray *et al.*, 2001) as sequence comparison of the SEBOV strain Boniface used in this study, compared to the SEBOV strain used by Bray *et al.* (2001), identified specific mutations in the L protein that may be associated with virulence and, hence, may explain the attenuation observed in the present study (S. Ibrahim and J. Farlow, personal communication). These observations highlight the need for consistent use of

viral isolates in studies aiming to identify anti-filovirus products for licensing.

The susceptibility of mice to airborne infection with filoviruses has, to our knowledge, not been previously demonstrated and our data suggested that comparable challenge doses of filoviruses may be equally lethal when delivered by either aerosol or IP routes. IP infections, however, lead to a more rapid progression of disease with reduced time to death. These data are consistent with the idea that filoviruses grow less readily in respiratory tract tissues and, therefore, may provide a possible explanation for the epidemiological observation that in human outbreaks of filovirus disease, infection is rarely transmitted via an airborne route. There is anecdotal evidence, however, to suggest that REBOV may have spread via aerosol transmission within a primate holding facility (Jaax *et al.*, 1995), and experimental non-human primates are certainly susceptible to infection by low numbers of filoviruses in aerosol form (Johnson *et al.*, 1995).

Further characterization of aerosol transmission in mouse models of infection with MARV or ZEBOV was undertaken by performing pathogenesis studies. Pathological observations were similar to those seen in mice following challenge studies with adapted viruses (Bray *et al.*, 1998; Warfield *et al.*, 2009) and in non-human primates exposed

Fig. 4. Histopathology of organ tissues from A129 IFN- α/β $-/-$ mice at different time points post-infection with MARV via the aerosol route and from uninfected mice. Tissues were stained using H&E stain. A129 IFN- α/β $-/-$ mice were exposed to a retained dose of $10 \text{ TCID}_{50} \text{ ml}^{-1}$ aerosolized MARV. (a) Liver tissue at day 2 post-challenge showing necrotic foci (arrow). (b) Spleen tissue at day 9 post-challenge showing lymphoid depletion (arrow). Control liver (c), spleen (d) and lung (e) tissues within normal limits from non-infected mice.

to wild-type viruses via various routes of transmission (Jaax *et al.*, 1995). There is limited information regarding the pathology of MARV or ZEBOV in human cases but multifocal necrosis of the liver and congestion and oedema in the lungs appear to be characteristic pathological features in human cases of infection (Kuhn, 2008), both of which were features in infected A129 IFN- α/β $-/-$ mice. These data confirm the aetiology of the disease as was shown by the exposure of the mice to the infectious viral strains.

The use of KO mice used in this study enabled us to distinguish between the relative lethality of different strains of filovirus and, to some extent, reflected the severity of disease associated with these strains in humans. The results of this study add to previous data by the use of different filovirus strains and the investigation of aerogenic infection of mice. A129 IFN- α/β $-/-$ mice clearly retain some ability to resist filovirus infection and, based on present evidence, may prove to be a useful preliminary model in which to investigate medical countermeasures prior to testing in primates and may be applicable for licensing.

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