The role of the Type I interferon response in the resistance of mice to filovirus infection

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Adult immunocompetent mice inoculated with Ebola (EBO) or Marburg (MBG) virus do not become ill. A suckling-mouse-passaged variant of EBO Zaire '76 ('mouse-adapted EBO-Z') causes rapidly lethal infection in adult mice after intraperitoneal (i.p.) inoculation, but does not cause apparent disease when inoculated subcutaneously (s.c.). A series of experiments showed that both forms of resistance to infection are mediated by the Type I interferon response. Mice lacking the cell-surface IFN-α/β receptor died within a week after inoculation of EBO-Z '76, EBO Sudan, MBG Musoke or MBG Ravn, or after s.c. challenge with mouse-adapted EBO-Z. EBO Reston and EBO Ivory Coast did not cause illness, but immunized the mice against subsequent challenge with mouse-adapted EBO-Z. Normal adult mice treated with antibodies against murine IFN-α/β could also be lethally infected with i.p.-inoculated EBO-Z '76 or EBO Sudan and with s.c.-inoculated mouse-adapted EBO-Z. Severe combined immunodeficient (SCID) mice became ill 3–4 weeks after inoculation with EBO-Z '76, EBO Sudan or MBG Ravn, but not the other viruses. Treatment with anti-IFN-α/β antibodies markedly accelerated the course of EBO-Z '76 infection. Antibody treatment blocked the effect of a potent antiviral drug, 3-deazaneplanocin A, indicating that successful filovirus therapy may require the active participation of the Type I IFN response. Mice lacking an IFN-α/β response resemble primates in their susceptibility to rapidly progressive, overwhelming filovirus infection. The outcome of filovirus transfer between animal species appears to be determined by interactions between the virus and the innate immune response.

Introduction

The filoviruses, Marburg and Ebola (MBG and EBO), cause the most severe viral haemorrhagic fevers of humans. In the first recorded MBG outbreak, the case fatality rate was 37%, while in two large hospital-associated epidemics in Zaire in 1976 and 1995, Ebola Zaire virus (EBO-Z) caused case fatality rates approaching 90% (Siegent, 1973; Pattyn et al., 1977; Sanchez et al., 1995). The natural reservoir of these viruses has not been identified. Transmission occurs by means of body fluids during close contact. Fever and intractable shock develop 1–2 weeks after exposure. Although some vaccines and antiviral therapies are effective in animals, none are approved for use in humans (Bray & Huggins, 1998; Sullivan et al., 2000).

Several factors contribute to the remarkable virulence of the filoviruses. Both MBG and EBO initially infect cells of the monocyte/macrophage lineage and then spread to many other cell types, including hepatocytes, fibroblasts and endothelial cells (Zaki & Goldsmith, 1998). Rapid dissemination of virus results in extensive cell lysis, accompanied by strong cytokine responses and coagulopathy, leading to shock and death (Peters et al., 1996). Filovirus disease thus has three phases: a silent incubation period, during which the virus replicates in its primary target cells and begins to disseminate; symptomatic illness, with fever, haemorrhage and shock; followed, in those victims that survive infection, by elimination of the virus by antigen-specific immune responses. In fatal cases, the first two phases of illness apparently proceed so rapidly that specific immune responses do not have time to come into play. The best opportunity for successful therapy may therefore be to develop medical interventions that prolong the incubation period, providing time for the host to assemble specific antigen-based immune responses.

Resistance to the initial phase of viral infection is mediated principally by the synthesis and secretion of Type I interferon (IFN), consisting of multiple variants of IFN-α and single forms of IFN-β and IFN-ω, which share a common cell-surface...
receptor (Meager, 1998). IFN-α is released from infected monocytes, macrophages and dendritic cells, while IFN-β is produced by a range of other cell types. [The other class of IFN, type II, or IFN-γ, has an unrelated sequence, receptor and function.] Type I IFN has a ‘primordial role…as a tightly regulated response system functioning predominantly in early antiviral defence’ (Müller et al., 1994). Its synthesis and secretion are triggered by the presence of intracellular double-stranded RNA and other signs of viral infection. The binding of IFN-α/β to the cell-surface receptor activates a cascade of signal transduction and transcription (Stat) proteins, leading to the transcription of a set of normally silent genes and the synthesis of 2′–5′ oligoadenylate synthetase (OAS), double-stranded-RNA-associated protein kinase (PKR), IFN regulatory factor 1 and other proteins, creating an ‘antiviral state’. The essential role of this response in antiviral defence is demonstrated by the sensitivity to lethal infection of knockout (KO) mice lacking the cell-surface receptor or the Stat1 protein, and of mice treated with antibodies to IFN-α (Gresser et al., 1976; Müller et al., 1994; Durbin et al., 1995). In mice, the Type I IFN response includes expression of the protein Mx1, which plays a role in resistance to a variety of RNA virus infections (Thimme et al., 1995; Haller et al., 1998). This gene is defective in most inbred strains, including BALB/c. Transgenic Mx1-negative mice express the corresponding human gene, MxA, are also resistant to a range of viruses (Frese et al., 1996).

A number of viruses have apparently acquired means of subverting or evading the Type I IFN response as part of their replication strategy (Katze, 1992; Meager, 1998). In the case of EBO-Z, it has been found that virus-infected human endothelial cells lose the ability to synthesize IFN-α in the presence of intracellular double-stranded RNA (Harcourt et al., 1998). A second study demonstrated that such cells were also unable to respond appropriately to exogenous IFN-α (Harcourt et al., 1999). Other investigators showed that cells expressing human MxA, although resistant to a number of RNA viruses, did not restrict the replication of filoviruses (Frese et al., 1996). Recent findings indicate that EBO-Z VP35 may be the viral protein responsible for suppression of the Type I IFN response (Basler et al., 2000). Subversion of antiviral mechanisms, denying the host time to recruit a specific immune response, together with the rapid replication rate, broad range of cell types infected and the lytic nature of the infection, may adequately explain the lethality of EBO-Z infection.

Filovirus infections have been studied in a number of animal models. EBO-Z, EBO Sudan and some MBG viruses cause febrile illness in guinea pigs and have been adapted to lethal virulence through a small number of animal-to-animal passages (Bowen et al., 1977; Ryabchikova et al., 1998). All MBG and EBO viruses cause lethal haemorrhagic fever in nonhuman primates (NHPs) (Bowen et al., 1978a; Peters et al., 1996). By contrast, MBG caused lethal illness when injected intracerebrally (i.c.) into newborn mice, but did not produce disease in older animals (Hofmann & Kunz, 1970; Siegert, 1973).

Similarly, EBO-Z '76 caused fatal infection when inoculated i.c., intraperitoneally (i.p.) or subcutaneously (s.c.) into newborn, but not weanling mice (Pattyn et al., 1977; Bowen et al., 1977; Van der Groen et al., 1979).

The latter findings appeared to provide no useful role for mice as an animal model of filovirus infection. However, progress in antiviral therapy eventually created a need to test new experimental drugs in a convenient small animal model. Initial studies showed that EBO-Z '76 caused lethal illness in severe, combined immunodeficient (SCID) mice, but the disease differed markedly from that in humans and NHPs in that the mice died 4–6 weeks post-challenge after a slow downhill course (Huggins et al., 1995). This consideration was the major stimulus for the attempt to adapt EBO-Z '76 to adult immunocompetent mice ('normal mice') through sequential passage in progressively older suckling mice (Bray et al., 1998). The resulting virus ('mouse-adapted EBO-Z'), causes rapidly lethal illness after i.p., but not s.c. inoculation, with a pattern of infection in the liver and spleen similar to that in EBO-Z-infected guinea pigs, NHPs and humans (Jaax et al., 1996; Bray et al., 1998; Ryabchikova et al., 1998; Zaki & Goldsmith, 1998; Connolly et al., 1999). The mouse model is now in use for antiviral drug and vaccine testing (Vanderzanden et al., 1998; Bray et al., 2000; Wilson et al., 2000).

As noted, normal adult BALB/c mice display two unique types of resistance to filovirus infection. First, they show no signs of illness after inoculation of viruses that cause disease in guinea pigs and primates. Second, their susceptibility to mouse-adapted virus injected i.p. and resistance to the same virus inoculated s.c. is a phenomenon lacking a counterpart in guinea pigs or primates. The present study employed a collection of EBO and MBG viruses and a range of immunocompetent, immunodeficient and gene-knockout (KO) mice, as well as normal mice treated with antibodies against murine IFN-α/β, to study the role of the Type I IFN response in these two forms of resistance to filovirus infection.

Methods

- **Viruses and cells.** Infectious material and animals were handled in maximum-containment biological safety level 4 (BSL-4) facilities at the United States Army Medical Research Institute of Infectious Diseases, Frederick, MD (USAMRIID). Laboratory personnel wore positive-pressure protective suits equipped with high-efficiency particulate air filters and supplied with umbilical-fed air.

- **The derivation of mouse-adapted EBO-Z has been described (Bray et al., 1998). The following viruses, amplified in Vero cells, were provided by Peter Jahrling, USAMRIID: EBO-Z from the 1976 and 1995 Zaire outbreaks ('EBO-Z '76, EBO-Z '95'); the Boneface strain from the 1976 Sudan outbreak (EBO Sudan); EBO Reston from a lethally infected monkey in the 1989 outbreak; EBO Ivory Coast from a human infection in 1994 (EBO IC); MBG Musoke (‘MBG Mus’) from a human case in Africa; the same virus serially passaged four times in guinea pigs (‘MBG Mus GP’); and MBG Ravn recovered from another human case, passaged twice in guinea pigs.

- **Normal, SCID and KO mice.** Newborn litters of BALB/c mice, adult female BALB/c mice and adult female SCID mice on a BALB/c...
Resistance of mice to filovirus infection

Results

Susceptibility of suckling mice to lethal EBO-Z infection.

The virulence of EBO-Z '76 and mouse-adapted EBO-Z was tested in 4-, 8-, 15- and 21-day-old mice. EBO-Z '76 was lethal by the i.p. route only for 4-day-old mice, and did not cause fatal infection when inoculated s.c. in any of these mice. By contrast, mouse-adapted EBO-Z was lethal for all mice when given by the i.p. route. It caused lethal infection when inoculated s.c. in all 4- and 8-day-old, and in 20% of 15-day-old mice, but 21-day-old mice were resistant to the virus.

Filovirus infection in IFN-α/β R −/− and Stat1 −/− mice

A collection of EBO and MBG viruses was used to challenge normal and KO mice. Normal (R +/+ ) strain 129 mice did not become ill after i.p. inoculation with non-mouse-adapted filoviruses, but died 5–6 days after injection with mouse-adapted EBO-Z (Table 1). By contrast, most of the viruses were lethal for IFN-α/β R −/− mice. EBO-Z '76 and EBO Sudan caused death in 5–7 days, as did guinea pig-adapted variants of MBG Musoke and Ravn, while non-adapted MBG Musoke produced a slower illness, from which one mouse recovered. EBO Reston and EBO IC did not cause illness. Electron microscopy and immunohistochemistry revealed large amounts of replicating virus and viral antigen on day 5 or 6 in the liver and spleen of mice ill with EBO-Z '76 and Sudan and MBG Musoke and Ravn, but not with EBO Reston or IC. Unexpectedly, EBO-Z '95 caused illness (diminished activity, ruffled fur) beginning on days 4–5, but was not lethal; EBO antigen was detected in scattered macrophages in the liver and spleen of a mouse killed on day 6. All R −/− mice that survived infection with EBO-Z '95, Reston or IC were protected against subsequent challenge with mouse-adapted EBO-Z, but they succumbed to a later challenge with MBG Ravn (not shown). All R +/+ mice that underwent silent infection with EBO viruses were protected against rechallenge with mouse-adapted EBO-Z. By contrast, all 15 R +/+ mice previously exposed to MBG viruses became ill after challenge with mouse-adapted EBO-Z, and all but two died.

Normal adult BALB/c mice developed lethal illness after i.p., but not s.c. challenge with mouse-adapted EBO-Z, and were not killed by EBO-Z '76 given by either route (Table 2). By contrast, IFN-α/β R −/− mice developed fatal illness after i.p. or s.c. inoculation with either virus. For a given route of
Table 1. Survival of strain 129 mice possessing or lacking the IFN-α/β receptor

Strain 129 mice (8–16 weeks old), possessing (+/+) or lacking (−/−) the IFN-α/β receptor, were first inoculated i.p. with 1000 p.f.u. of the filovirus listed at left (‘Challenge 1’), and then on day 18 post-infection were inoculated i.p. with 1000 p.f.u. of mouse-adapted EBO-Z (‘Rechallenge’). Surv., survival; MTD, mean time (days) to death of those mice that died.

A single s.c. inoculation of anti-murine-IFN-α/β antibodies on filovirus infection

inoculation, mouse-adapted EBO-Z was more rapidly lethal than EBO-Z ’76, while for a given virus, the i.p. route produced more rapid death than s.c. injection. The pattern of viral antigen distribution in the liver and spleen of IFN-α/β R −/− mice infected with EBO-Z ’76 was indistinguishable from that following i.p. infection of mouse-adapted EBO-Z in normal mice (not shown; Bray et al., 1998). Stat1 −/−/− mice were also susceptible to lethal infection with s.c.-injected mouse-adapted EBO-Z or with i.p.-injected EBO-Z ’76 (Table 2).

Table 2. Survival of normal BALB/c mice, or strain 129 mice lacking the IFN-α/β receptor or the Stat1 protein

Normal BALB/c mice, or strain 129 mice lacking the IFN-α/β receptor or the Stat1 protein (8–16 weeks old) were inoculated i.p. or s.c. with the indicated viruses. Surv., survival; MTD, mean time to death (days).

Effect of anti-IFN-α/β antibodies on filovirus infection in normal BALB/c mice

A single s.c. inoculation of anti-murine-IFN-α/β antibodies at the time of challenge made normal adult BALB/c mice susceptible to rapidly lethal infection with i.p.-inoculated EBO-Z ’76 or EBO Sudan (Fig. 1A). Typically, antibody-treated mice began losing weight on day 3 post-infection, and most were dead by day 8. Antibodies alone caused no weight loss or illness. Antibody-treated mice did not develop illness after i.p. inoculation of EBO Reston or EBO-Z ’95. In contrast to IFN-α/β R −/− mice, they also did not become ill after injection with MBG Musoke or Rav. Inoculation of EBO-Z ’76 plus a one-fourth dose of antibodies caused about half as much weight loss and no deaths, while a one-eighth dose produced no effect. Antibody treatment on day 0 or day 2 made mice susceptible to i.p. infection with EBO-Z ’76, but treatment on day 4 had no effect (Fig. 1B). Large amounts of viral antigen were present in the livers and spleens of antibody-treated mice.
Fig. 1. Weight loss and survival of filovirus-infected normal BALB/c mice treated with anti-IFN-α/β antibodies. (A) Percentage change in weight of groups of four adult mice inoculated i.p. with EBO-Z ’76, EBO-Z ’95, EBO Sudan or EBO Reston and s.c. with anti-IFN-α/β antibodies, or i.p. with mouse-adapted EBO-Z. (B) Percentage change in weight of mice inoculated i.p. with EBO-Z ’76 on day 0 and s.c. with antibodies on day 0, 2 or 4. (C) Percentage survival of groups of mice injected s.c. with 100 p.f.u. of mouse-adapted virus on day 0 and with anti-IFN-α/β antibodies on day 2, 4, 6 or 8, or left untreated. Result for day 0 (not shown) resembled that for day 2.

Fig. 2. Filovirus infection of SCID mice. (A) Percentage change in body weight of groups of six adult SCID mice inoculated i.p. with 1000 p.f.u. of mouse-adapted EBO-Z or with EBO-Z ’76, EBO Sudan or guinea pig-adapted MBG Ravn suspended in EMEM. Results of inoculation with EBO-Z ’95, EBO Reston, MBG Musoke, guinea pig-adapted MBG Musoke (not shown) resembled those for mice injected with EMEM only (Xs). (B) Survival of adult SCID mice inoculated i.p. or s.c. with 100 p.f.u. of mouse-adapted EBO-Z or EBO-Z ’76 and observed for 40 days. (C) Percentage change in weight of groups of six adult SCID mice inoculated i.p. with 100 p.f.u. of EBO-Z ’76 and s.c. with anti-IFN-α/β antibodies on day 0 or 5, or s.c. with placebo (EMEM) on day 0.

dying from non-mouse-adapted EBO-Z ’76, but none was detected in infected mice not treated with antibodies.

Antibody treatment also rendered normal mice susceptible to lethal infection with s.c.-inoculated mouse-adapted EBO-Z, but not EBO-Z ’76. Treatment of s.c.-injected mice could be delayed much longer than in the case of i.p. infection: all mice treated on day 0, 2 or 4 died from infection, while treatment on day 6 resulted in the death of 50%, and treatment on day 8 in the death of 25% of the animals (Fig. 1C). Treatment on day 10 had no effect.
Effect of anti-IFN-α/β antibodies on filovirus infection in SCID mice

The role of endogenous IFN-α/β in determining the time-course of illness in filovirus-infected SCID mice was also examined. I.p.-inoculated EBO-Z ‘76, EBO Sudan and guinea pig-adapted MBG Ravn caused severe illness and death, with weight loss commencing 14–20 days after i.p. challenge (Fig. 2A). Mouse-adapted EBO-Z, by contrast, caused the death of all mice by day 5 when inoculated i.p., and by day 12 when injected s.c. (Fig. 2B). The route of inoculation had no apparent effect on the length of the disease course caused by EBO-Z ‘76.

The disease was sharply accelerated if viral challenge was accompanied by an injection of anti-IFN-α/β antibodies (Fig. 2C). In the experiment shown, mice given EBO-Z ‘76 plus placebo on day 0 did not begin to lose weight for almost 3 weeks, and the first death did not occur until day 40. By contrast, antibody treatment on day 0 resulted in the death of all mice by day 6. Antibody treatment to day 5 resulted in the abrupt onset of weight loss on day 7, from which all mice partially recovered. Antibodies alone had no effect.

Effect of anti-IFN-α/β antibodies on antiviral therapy in normal BALB/c mice

It was previously shown that a single injection of an adenosine analogue, c3-Npc A, protected mice against challenge with mouse-adapted EBO-Z (Bray et al., 2000). In order to determine whether the innate antiviral response contributed significantly to this successful outcome, virus-infected adult BALB/c mice were treated with c3-Npc A on day 0, 1 or 2, with or without an accompanying s.c. injection of anti-IFN-α/β antibodies. Two experiments showed that mice treated with drug and placebo on day 0 or 1 maintained their body weight and survived infection, but those that received both drug and antibodies lost weight as fast as untreated controls, and were dead by day 7 (Fig. 3A, B). However, all mice treated on day 2 maintained their weight and survived, whether or not they were treated with antibodies.

Discussion

It was initially assumed that the resistance of normal adult mice to filovirus infection resulted from an inability of these viruses to infect murine cells. However, mice deprived of their endogenous IFN-α/β response proved to be susceptible to lethal infection by a number of filoviruses. EBO-Z ‘76, EBO Sudan, MBG Musoke and MBG Ravn produced a disease in IFN-α/β R−/− mice that was almost as rapidly fatal as that caused by mouse-adapted EBO-Z in normal mice. Pathology studies revealed typical features of disseminated filovirus infection, ruling out involvement of a contaminating agent. Normal adult BALB/c mice treated with anti-IFN-α/β antibodies were also susceptible to lethal infection with EBO-Z ‘76 and EBO Sudan.

There proved to be a ‘gradient of virulence’ for filoviruses, as shown in Table 3. EBO-Z ‘76 and EBO Sudan were lethal for SCID, IFN-α/β R−/− and antibody-treated normal mice, while EBO Reston and IC caused no apparent illness. Interestingly, EBO Reston, and perhaps EBO IC, also appear to be less virulent for humans. The molecular basis of this diminished virulence is still undefined. EBO-Z ‘95, which is as lethal for humans as EBO-Z ‘76, unexpectedly did not cause death in R−/− or SCID mice. Since the complete sequence of both viruses has been determined, and mouse-adapted EBO-Z and other viruses are currently being sequenced, it may soon be possible to identify specific loci responsible for differences in virulence. As expected, based on sequence divergence between EBO and MBG viruses, IFN-α/β R−/− mice exposed to EBO Reston or IC were protected against later challenge with mouse-adapted EBO-Z, suggesting that a live, attenuated filovirus vaccine would also be effective in primates.
Four-day-old virus-infected mice produced more IFN-αβ-confer resistance to infection in cell culture (Frese et al. 1996; Haller et al., 1998; Hefti et al., 1999). It is conceivable that Mx1-positive mice will demonstrate a measurable degree of resistance to challenge with mouse-adapted EBO-Z, compared to Mx1-negative inbred strains. On the other hand, since MxA does not protect humans against filoviruses, and did not confer resistance to infection in cell culture (Frese et al., 1996), it is unlikely that MxA would provide protection when inoculated EBO-Z ‘76 was irreversibly suppressed by day 4.

In contrast to the essential role of the Type I IFN response in preventing the breakthrough of infection from initial sites of replication, antigen-specific immune responses appear to be required to ‘clean up’ and eliminate infection. This is well illustrated by the fact that SCID mice remained healthy for several weeks after infection with EBO-Z ‘76, but died within days if deprived of their IFN-αβ response by antibody treatment, as did R/−/− mice inoculated with the same virus. However, the fact that SCID mice survived infection with EBO Reston, EBO IC and EBO-Z ‘95, as well as with some MBG viruses that killed R/−/− mice, indicates that specific immune responses may not be needed to eliminate some of the less virulent agents.

As noted, the specific mutation(s) responsible for the difference in virulence for mice between mouse-adapted EBO-Z and other filoviruses has not yet been defined. The present study suggests that selection during serial passage favoured a variant that was able to suppress or evade the Type I IFN response. Other investigators have recently provided evidence that the EBO-Z VP35 protein may be responsible for such suppression in primates (Basler et al., 2000). The enhanced virulence of mouse-adapted EBO-Z for mice may be the result of a mutation in VP35 or another viral protein that altered the virus’s relationship with the innate immune response, changing it from one lethal only for primates to one that is also lethal for mice. Recent studies have shown that the mouse-adapted virus may, in fact, be somewhat attenuated for NHPs, perhaps as a result of the same mutation (M. Bray and others, unpublished data). Sequencing of the genome of mouse-adapted EBO-Z and its non-mouse-adapted precursor is currently in progress.

Because of the strong innate antiviral responses to filovirus infection in rodents, vaccines and antiviral drugs that are protective in rodent models may prove to be less efficacious in primates. This potential bias could be avoided by performing initial testing in mice lacking a Type I response. In particular, since R/−/− mice have normal B- and T-cell-mediated immune responses, they could be used to define the relative roles of innate, humoral and cellular immunity in resistance to infection, as shown for other viruses (Muller et al., 1994; Van den Broek et al. 1995a, b). As shown in this study, mice deprived of an IFN-αβ response can also help to clarify the

Table 3. Comparative virulence of filoviruses

<table>
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<tr>
<th>Normal BALB/c</th>
<th>BALB/c+ anti-IFN</th>
<th>SCID</th>
<th>IFN-αβ R/−/−</th>
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<tr>
<td>EBO-Z ’76</td>
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<td>EBO-Z ’95</td>
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<td>EBO Sudan</td>
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<td>MBG Ravn GP</td>
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By contrast, all R/−/− mice that survived exposure to EBO viruses died after later challenge with MBG. All 15 R/+/+ mice that were first exposed to MBG became ill when challenged with mouse-adapted EBO-Z, and all but two died. Given the combined evidence of the absence of cross-protection between MBG and EBO, the survival of these two mice may simply have been the result of chance.

This study also delineated a ‘gradient of susceptibility’ for mice, with IFN-αβ R/−/− mice being subject to lethal infection by the greatest number of filoviruses (Table 3). Such mice resemble humans in their susceptibility to multiple EBO and MBG viruses and in their resistance to EBO Reston. Studies using other types of mice – such as outbred mice expressing functional Mx1, inbred Mx1-negative mice expressing human MxA and KO mice lacking PKR, OAS or other ‘antiviral’ proteins – will help to define the precise mechanisms of susceptibility and resistance to filovirus infection (Thimme et al., 1995; Haller et al., 1998; Hefti et al., 1999). It is conceivable that Mx1-positive mice will demonstrate a measurable degree of resistance to challenge with mouse-adapted EBO-Z, compared to Mx1-negative inbred strains. On the other hand, since MxA does not protect humans against filoviruses, and did not confer resistance to infection in cell culture (Frese et al., 1996), it is unlikely that MxA would provide protection when expressed in mice.

The acquired resistance of suckling mice to EBO-Z infection with increasing age has been observed for other viruses. A pattern almost identical to that described here occurs in Sindbis virus-infected suckling CD-1 mice (Trgovcich et al., 1999). Four-day-old virus-infected mice produced more IFN-α than 8-day-old mice in response to viral infection, but the cells of older mice were more responsive to IFN-α. Similarly, Pfeifer et al. (1993) found that the expression of Z' -apos; OAS induced by IFN-α increased with age, reaching its maximum in adult mice.

The same factors presumably play an important role in the acquired resistance of normal mice to filovirus infection.

The present study showed that the Type I IFN response also plays a key role in the resistance of normal mice to s.c.-inoculated mouse-adapted EBO-Z. The virus clearly interacts in very different ways with innate immune mechanisms when inoculated by the i.p. and s.c. routes; IFN-α is required for this process, which may depend on IFN-activated natural killer cells. Remarkably, treatment with anti-IFN-α/β antibodies precipitated illness even when delayed to day 8 after s.c. infection, indicating that the virus remained in some way sequestered, perhaps in regional lymph nodes. By contrast, i.p.-inoculated EBO-Z ’76 was irreversibly suppressed by day 4.

In contrast to the essential role of the Type I IFN response in preventing the breakout of virus from initial sites of replication, antigen-specific immune responses appear to be required to ‘clean up’ and eliminate infection. This is well illustrated by the fact that SCID mice remained healthy for several weeks after infection with EBO-Z ’76, but died within days if deprived of their IFN-α/β response by antibody treatment, as did R/−/− mice inoculated with the same virus. However, the fact that SCID mice survived infection with EBO Reston, EBO IC and EBO-Z ’95, as well as with some MBG viruses that killed R/−/− mice, indicates that specific immune responses may not be needed to eliminate some of the less virulent agents.

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Because of the strong innate antiviral responses to filovirus infection in rodents, vaccines and antiviral drugs that are protective in rodent models may prove to be less efficacious in primates. This potential bias could be avoided by performing initial testing in mice lacking a Type I response. In particular, since R/−/− mice have normal B- and T-cell-mediated immune responses, they could be used to define the relative roles of innate, humoral and cellular immunity in resistance to infection, as shown for other viruses (Muller et al., 1994; Van den Broek et al. 1995a, b). As shown in this study, mice deprived of an IFN-αβ response can also help to clarify the
mechanism of action of antiviral drugs. In this regard, adenosine analogues that inhibit cellular SAH hydrolase are of particular interest, since even single doses can cure lethal EBO-Z infection in mice (Bray et al., 2000). These compounds are thought to block virus replication by preventing intracellular methylation reactions, including that of the 5′ cap of viral messenger RNA. However, such a general metabolic alteration clearly may also result in other changes in the host. The effect of anti-IFN-α/β antibodies in eliminating the effectiveness of c5-Npc A given on day 0 or 1 suggests that the drug either induces a strong IFN-α/β response, or else acts through a different mechanism that is only adequate to bring about survival when the Type I IFN response is intact. The reproducible failure of antibody treatment to block the action of the drug when given on day 2 is unexplained; these phenomena are the subject of current investigation. SAH hydrolase inhibitors have proven to be less effective in NHPs than in mice (M. Bray and others, unpublished data). Understanding the basis of their strong activity in mice may help to improve this approach to therapy.

Mice can also be cured of mouse-adapted EBO-Z infection by treatment with native murine or recombinant IFN-α, providing therapy is initiated soon after challenge (M. Bray and others, unpublished data). This indicates that mouse-adapted EBO-Z is able to overcome any initial barriers to infection of its primary target cells, but cannot effectively replicate in cells in which IFN-α has already induced an ‘antiviral state’. In NHPs, by contrast, IFN-α treatment beginning on the day of challenge was far less effective, resulting in at most a 1 day delay in the onset of illness and death (Bowen et al., 1978b; Jahrling et al., 1999). This suggests that in primates, filoviruses are able to overcome antiviral mechanisms in cells already affected by IFN-α. One goal of further research should therefore be to understand the mechanisms by which filoviruses are so efficiently suppressed in mice, to aid in the development of more effective countermeasures for humans.

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References


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