Disease modeling for Ebola and Marburg viruses

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The filoviruses Ebola and Marburg are zoonotic agents that are classified as both biosafety level 4 and category A list pathogens. These viruses are pathogenic in humans and cause isolated infections or epidemics of viral hemorrhagic fever, mainly in Central Africa. Their natural reservoir has not been definitively identified, but certain species of African bat have been associated with Ebola and Marburg infections. Currently, there are no licensed options available for either treatment or prophylaxis. Different animal models have been developed for filoviruses including mouse, guinea pig and nonhuman primates. The ‘gold standard’ animal models for pathogenesis, treatment and vaccine studies are rhesus and cynomolgus macaques. This article provides a brief overview of the clinical picture and the pathology/pathogenesis of human filovirus infections. The current animal model options are discussed and compared with regard to their value in different applications. In general, the small animal models, in particular the mouse, are the most feasible for high biocontainment facilities and they offer the most options for research owing to the greater availability of immunologic and genetic tools. However, their mimicry of the human diseases as well as their predictive value for therapeutic efficacy in primates is limited, thereby making them, at best, valuable initial screening tools for pathophysiology, treatment and vaccine studies.

Research problem

Ebola virus (EBOV) and Marburg virus (MARV) infections cause a severe form of viral hemorrhagic fever (VHF) with lethality in humans ranging from 23-90% depending on the virus species and strain (Sanchez et al., 2007). Given the high virulence in humans and the classification of these viruses as biosafety level 4 and category A list pathogens, animal models are crucial for understanding the underlying mechanisms of disease, as well as for the development of therapeutics and vaccines. Furthermore, for biothreat agents, where studies of clinical effectiveness in humans are impossible, the new FDA animal rule comes into place when demonstrating efficacy in animal models that resemble human cases and for which the natural history of the particular disease is fully understood [http:www.fda.gov/cder/guidance/8324concept.pdf].

The ‘gold standard’ animal model for EBOV and MARV is the nonhuman primate (NHP) because these are the only animals that are lethally infected with non-adapted human isolates and the resulting pathology is close to that described in humans (Sanchez et al., 2007). However, owing to ethical, practical and expense issues in dealing with NHPs, small animal models have had to be created. Small animal models have been developed for Zaire ebolavirus (ZEOBV) and Lake Victoria marburgvirus (LVMARV) through serial passaging in both mice and guinea pigs. This adaptation process is necessary because wild-type virus induces no discernible pathology in these small animals.

The pathogenesis of the adapted rodent strains differs in many aspects but similarities to the disease in primates have also been demonstrated (Table 1). Nevertheless, the rodent models are the first choice for in vivo, efficacy screening studies for therapeutics and vaccines. However, their predictive value for efficacy in primates is limited (Geisbert et al., 2002). There are currently no adequate small animal models for the remaining EBOV species [Sudan ebolavirus (SEBOV), Cote d’Ivoire ebolavirus (CIEBOV) and Reston ebolavirus (REBOV)] (Feldmann et al., 2005a).

Filoviral hemorrhagic fever

Clinical presentation

Following an incubation period of 2-21 days, human EBOV and MARV infections normally show an abrupt disease onset that is characterized by flu-like symptoms (fever, chills, malaise and myalgia). The subsequent signs and symptoms indicate multi-system involvement, including systemic (prostration, lethargy), gastrointestinal (anorexia, nausea, vomiting, abdominal pain, diarrhea), respiratory (chest pain, shortness of breath, cough), vascular (conjunctival infection, postural hypotension, edema) and neurologic (headache, confusion, seizure, coma) manifestations. Hemorrhagic manifestations may develop during the peak of the illness and include petechiae, ecchymoses, uncontrolled bleeding from venipuncture sites, epistaxis and other mucosal hemorrhages, and post-mortem evidence of visceral hemorrhagic effusions. In addition, there is often a maculopapular rash associated with varying degrees of erythema and desquamation. In late stages of the disease, shock, convulsions, severe metabolic disturbances and diffuse coagulopathy occur. Fatal cases develop clinical signs early during infection and demise typically occurs in the second week, mainly as a result of the consequences of hypovolemic shock. Fever is present in nonfatal cases for about 5-9 days and improvement typically coincides with when

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the antibody response is noted (days 7-11). Convalescence is prolonged and sometimes associated with myelitis, recurrent hepatitis, psychosis or uveitis (for reviews, see Martini and Siegert, 1971; Pattyn, 1978; Peters and LeDuc, 1999; Feldmann et al., 2003; Sanchez et al., 2007).

**Pathogenesis**

In general terms, human VHF resulting from EBOV and MARV infections is associated with fluid distribution problems, hypotension and coagulation disorders, and often leads to fulminant shock and subsequent multiorgan system failure (Fig. 1). Viral replication, in conjunction with immune and vascular dysregulation, is thought to play a role in disease development. Specific organ involvement includes extensive disruption of the parafollicular regions in the spleen and lymph nodes, and proliferation of the virus in mononuclear phagocytic cells has been demonstrated. A dramatic lymphopenia is thought to be the result of ‘bystander apoptosis’, most likely triggered through either mediators released from virus-activated primary target cells or by as yet unidentified interactions between host and viral products. In contrast to the activation of monocytes/macrophages, infected dendritic cells were impaired in the secretion of pro-inflammatory cytokines, the production of co-stimulatory molecules and the stimulation of T cells. The ability of filoviruses to interfere with the host innate immune system, especially the interferon (IFN) response, has been attributed to the virion proteins (VP) 35 and VP24.

Overall, EBOV and MARV infections clearly affect the innate immune response but with obvious varying outcomes. In particular, the presence of IL-1β and elevated levels of IL-6 during the early symptomatic phase of the disease have been suggested as blood markers for survival, whereas the release of IL-10 and high levels of neopterin deposits were found in mouse tissues (Bray et al., 1998). The pathogenesis of infection (innate immune response), particularly the type 1 IFN response (Bray et al., 2001) (Tables 1 and 2). Newborn mice, however, succumb to lethal infection following intraperitoneal or intracerebral infection (van der Groen et al., 1979), which might be explained by an incompletely developed type I IFN response in these mice (Pfeifer et al., 1993). Filovirus infection is also lethal for adult immunodeficient mice such as the severe combined immunodeficient (SCID) mouse, which lacks functional B- and T-cell responses (Table 2). Unlike humans or other animal models, SCID mice remain healthy for several weeks, then develop gradual, progressive weight loss and slowing of activity, and then die 20-25 or 50-70 days after ZEBOV or MARV challenge, respectively (Bray, 2001; Warfield et al., 2007). Mice lacking a complete type 1 IFN response (innate immune response), such as knockout mice that do not express STAT or the IFN receptor α/β, uniformly die within a week of subcutaneous challenge with a variety of filovirus strains (Bray, 2001) (Table 2).

An immunocompetent murine model was developed by passaging ZEBOV nine times in progressively older suckling mice (Bray et al., 1998) (Tables 1 and 2). In mice infected with this mouse-adapted strain, the onset of illness was 3 days after inoculation and death occurred after 5-7 days. High virus titers [up to 10⁹ plaque-forming units (PFU)/gram] could be detected in the liver and spleen (Bray et al., 1998). The pathological changes in the liver and spleen, as well as the levels of serum transaminases (aspartate transaminase, AST; alanine transaminase, ALT), in infected mice resembled those in ZEBOV-infected primates (Bray et al., 2001). However, in contrast to primate infections, only a few fibrin deposits were found in mouse tissues and infection was only lethal when the virus was inoculated intraperitoneally. Furthermore, mice were resistant to large doses of the same virus when inoculated by other routes.

At this time, no immunocompetent mouse model for MARV has been reported (Table 1). A recent report has described a SCID mouse model, which uses liver homogenates from MARV-infected SCID mice that have been serially passaged ten times, that reduces the time to death from between 50-70 days to 7-10 days (Warfield et al., 2007). At 3-4 days post-inoculation, infected mice showed weight loss, a hunched appearance and exhibited decreased grooming; some mice appeared to have hemorrhages and some developed hind-leg paralysis. The viremia peaked at around 10⁶ PFU/ml in serum at days 6-8. MARV was present at high titers in the
blood, liver, spleen, kidney and other organs. After infection, profound thrombocytopenia, as well as notable alterations in serum chemistry levels (especially liver enzymes), occurred with progressively increasing severity (Warfield et al., 2007).

**Guinea pig models**

Guinea pigs inoculated with wild-type EBOV develop only a short-lived, nonlethal febrile illness (Bowen et al., 1978; Bowen et al., 1980; Ryabchikova et al., 1996). A lethal guinea pig model for EBOV infection was developed eventually by infecting inbred and outbred guinea pig strains with ZEBOV, followed by sequential passages (4-8 times) of virus in naïve guinea pigs (Bowen et al., 1978; Bowen et al., 1980; Connelly et al., 1999; Ryabchikova et al., 1996) (Table 1). The resulting guinea-pig-adapted strains of virus were uniformly lethal, typically resulting in death after 8-11 days. Infected guinea pigs exhibit few clinical signs of infection until around day 5 when they cease eating and become febrile and dehydrated. In these guinea pigs, death was not accompanied by visible signs of hemorrhage (Ryabchikova et al., 1996). The virus was first detected in the spleen and liver on day 2, and by day 3 it could also be detected in the kidney, adrenal gland, lung and pancreas. Mean organ titers rose progressively and reached their highest levels (4.8-6.4 log_{10} PFU/g) on day 9, and rose progressively and reached their highest levels (4.8-6.4 log_{10} PFU/g) on day 9, and viremia peaked on day 7 with ~10^5 PFU/ml (Connolly et al., 1999). Infected guinea pigs also showed progressive prolongation of the prothrombin time (PT) and the partial thromboplastin time (aPTT).

As seen in ZEBOV infection, guinea pigs that were experimentally infected with wild-type MARV developed only a mild febrile disease and most of them survived the infection (Table 1). The virulence of the virus was increased by serial passage in guinea pigs. Four to eight passages were sufficient to produce a virus that was lethal for all animals by 7-17 days after infection (Simpson et al., 1968). Infected animals showed weight loss, elevated temperature and edematous faces, and the blood from some animals failed to clot. As seen in NHPs, a sudden decrease in temperature occurred shortly before death.

**Nonhuman primates**

Filovirus infections have been intensively investigated in various species of NHP, but mainly in cynomolgus (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*). African green monkeys (*Cercopithecus aethiops*) are resistant to REBOV and baboons (*Papio hamadryas*) appear to be somewhat more resistant to all EBOV species (Fisher-Hoch et al., 1992; Fisher-Hoch and McCormick, 1999; Gonchar et al., 1991; Ryabchikova et al., 1999a; Ryabchikova et al., 1999b). The viral dose and strain, the route of infection and the species of NHP used all appear to influence the onset, duration and severity of the clinical signs.

Several NHP species have been used as models to study ZEBOV infection, namely the African green monkey (Bowen et al., 1978; Fisher-Hoch et al., 1992; Davis et al., 1997; Ryabchikova et al., 1999a; Ryabchikova et al., 1999b), hamadryad baboon (Jones, 1980; Mikhailov et al., 1995; Borisevich et al., 1995; Ryabchikova et al., 1996; Kudoyarova-Zubavichene et al., 1999; Ryabchikova et al., 1999a; Ryabchikova et al., 1999b), rhesus macaque (Bowen et al., 1978; Fisher-Hoch et al., 1985; Piankov et al., 1995; Jaxx et al., 1996; Johnson et al., 1996; Geisbert et al., 2002) and cynomolgus macaque (Jones, 1980; Fisher-Hoch et al., 1992; Geisbert et al., 2003). For cynomolgus macaques infected with ZEBOV, the onset of clinical signs is fairly rapid, occurring within 4-5 days. In other NHP models, the onset of symptoms is slower and thus more similar to that observed in humans. Usually macaques become febrile and lethargic between 2-3 days after infection and fever persists throughout the course of the disease. A drop in body temperature usually precedes death. Animals also show weight loss of up to 10% of their body weight, which is probably primarily related to dehydration rather than mobilization of fat reserves and catabolism – although all of these factors probably con-
In addition, some animals develop diarrhea and intermittent melena. As soon as day 4 after infection, NHPs generally develop a maculopapular rash that remains prominent until death. Lymphadenopathy of peripheral lymph nodes develops early in the disease course and an enlarged liver with rounded capsular borders is seen at mid- to late-stages of the disease (Geisbert and Hensley, 2004). Virus can normally be detected in the blood on day 2 after infection and usually peaks 2-3 days later. Throughout the course of the disease, a rise in both absolute and relative neutrophil counts develops, coinciding with severe lymphopenia in which neutrophils account for over 90% of all leukocytes. In addition, a marked thrombocytopenia is uniformly seen in these infected animals. Prolongation of the aPTT has been reported as early as day 6 after infection and by day 10-12 blood samples often lose their ability to clot (Fisher-Hoch et al., 1983).

Plasma levels of sodium, potassium and calcium all fall during disease progression, whereas urea and creatinine levels increase. Further, the levels of the liver transaminases (AST, ALT) start to increase, usually around day 5, and remain high until death (Fisher-Hoch et al., 1983; Fisher-Hoch et al., 1985).

Table 1. Infection in animal models and humans

<table>
<thead>
<tr>
<th></th>
<th>Mouse (wt)</th>
<th>Mouse (ad)*</th>
<th>Guinea pig (wt)</th>
<th>Guinea pig (ad)</th>
<th>NHP (wt)</th>
<th>Human (wt)</th>
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<tbody>
<tr>
<td>Viremia</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Virulence</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Primary cell tropism</td>
<td>Unknown</td>
<td>MPC</td>
<td>Unknown</td>
<td>MPC</td>
<td>MPC</td>
<td>MPC</td>
</tr>
<tr>
<td>Macular rash</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Coagulation abnormalities</td>
<td>No</td>
<td>Not profound</td>
<td>No</td>
<td>Some</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Liver enzymes</td>
<td>Unknown</td>
<td>Elevated</td>
<td>Unknown</td>
<td>Elevated</td>
<td>Elevated</td>
<td>Elevated</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Unknown</td>
<td>Yes</td>
<td>Unknown</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Lymphocyte apoptosis</td>
<td>Unknown</td>
<td>Yes</td>
<td>Unknown</td>
<td>Limited</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Proinflammatory cytokine response</td>
<td>Unknown</td>
<td>Yes</td>
<td>Unknown</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

MPC = mononuclear phagocytic cell; ad = adapted strain; wt = wild-type strain; *Ebola virus only.

Table 2. Ebola virus infection in mice

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Mouse strain</th>
<th>ZEBOV (ad)(i.p.)</th>
<th>ZEBOV (ad)(s.c.)</th>
<th>ZEBOV (wt)(i.p.)</th>
<th>ZEBOV (wt)(s.c.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>BALB/c, C57BL/6</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Adaptive immune response</td>
<td>SCID</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Rag-2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Nude</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Beige</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IFN-γ knockout</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>TNF-α knockout</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Innate immune response</td>
<td>IFN-α/β-receptor knockout</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>STAT1 knockout</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Lymphotoxin-α knockout</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

+= lethal infection; -= no apparent disease; ad = adapted strain; i.p. = intraperitoneal; s.c. = subcutaneous; wt = wild-type strain [summarized from Bray (Bray, 2001)].
Clinical terms

Ecchymoses – bruise-like black-and-blue or purple skin lesions caused by ruptured blood vessels

Epistaxis – nosebleed

Viral hemorrhagic fevers (VHFs) – a diverse group of animal and human illnesses that are caused by RNA viruses, characterized by fever and bleeding disorders and can progress to high fever, shock and death in extreme cases

Hypovolemic shock – decreased blood volume causing insufficient blood circulation, also known as hypovolemia

Lymphadenopathy – abnormal swelling and enlargement of the lymph nodes, indicative of disease

Maculopapular rash – a flat, red skin rash covered with bumps, thus containing characteristics of both macules (flat, discolored regions) and papules (raised bumps)

Melana – black, tar-like stools associated with gastrointestinal bleeding

Myalgia – muscle pain

Petechiae – small reddish or purplish spots resulting from a localized hemorrhage in skin or mucous membrane

Thrombocytopenia – a persistent low blood platelet count

Viremia – presence of viruses in the blood

Clinical and basic research opportunities

- Creating small animal models of Sudan ebolavirus (SEBOV), Cote d’Ivoire ebolavirus (CIEBOV) and Reston ebolavirus (REBOV)
- Development of an immunocompetent mouse model for MARV
- Development of EBOV and MARV models in smaller non-human primate species
- In vivo screening studies for new EBOV and MARV therapeutics and vaccines
- Using animal models for clinical effectiveness studies in accordance with new guidelines set by the Food & Drug Administration (FDA) and Center for Biologics Evaluation and Research (CBER)

Discussion of animal models

Although rodent models have some similarities to the human disease, they are of limited value for clinical disease presentation of human filovirus infection because the disease course in rodents differs from that reported in humans and NHPs (Gibb et al., 2001; Geisbert et al., 2002; Feldmann et al., 2003). In addition, important clinical signs such as maculopapular rash and an elevated temperature throughout the course of the disease are missing. Mice do not display all of the characteristics of DIC, which is a hallmark of filovirus infection in primates that includes prolongation of PT and aPTT, circulating fibrin degradation products, decreased plasma fibrinogen and decreased fibrin deposition (Bray et al., 2001; Geisbert and Hensley, 2004; Sanchez et al., 2007). Compared with mice, infected guinea pigs develop more severe coagulation defects, including a drop in platelet counts and an increase in coagulation time, but the level of fibrin deposition and coagulopathy are not as high as the levels seen in NHPs (Connolly et al., 1999; Reed and Mohamadzadeh, 2007). Further, lymphocyte bystander apoptosis, an important feature in primates and mice, is not as prominent in guinea pigs (Bray et al., 1998; Connolly et al., 1999; Bradfute et al., 2007). Mice differ from guinea pigs and monkeys in that they display a decrease in blood urea nitrogen (BUN), rather than an increase (Bray, 2001). Additionally, the histopathologic features of filovirus disease in humans are more closely mirrored by NHPs than rodents models (Zaki and Goldsmith, 1999). NHPs are excellent models with which to study filovirus pathogenesis because they closely resemble the clinical disease and pathology described in humans. When selecting a suitable NHP model, the species, sex and age of the NHPs, together with the route of infection and the administration dose, must all be taken into consideration because all of these factors will have an influence on the study (Geisbert et al., 2004). Cynomolgus and rhesus macaques are considered the ‘gold standard’ models for filovirus infections, and studies using rhesus macaques have additional advantages in that this species is widely used in the pharmaceutical industry and its genome sequence has been published (Geisbert et al., 2004; Rhesus Macaque Genome Sequencing and Analysis Consortium, 2007). Nevertheless, the increase demand for NHPs for biodefense and infectious disease research has contributed to a current shortage of macaques (Cohen, 2000; Patterson and Carrion, 2005; Satkoski et al., 2008). In the future, the development of a model that uses a smaller species of NHP, such as certain species of new world monkeys, might help to ease the burden.

Despite the differences in clinical presentation and pathogenesis, rodents can serve an important role for the initial in vivo evaluation of filovirus vaccines and treatment schemes (Huggins et al., 1995; Wilson et al., 2000). In particular, mice offer certain advantages including the ease with which large numbers of animals can be obtained and the availability of numerous strains, including genetically engineered strains, as well as a wide range of well-characterized reagents. However, one must use caution when interpreting data from rodents, because a number of antiviral therapies and vaccines have been shown to be effective in rodents but then failed in NHP models (Bray and Paragas, 2002; Geisbert et al., 2002; Feldmann et al., 2005b; Reed and Mohamadzadeh, 2007). This might be the result of considerable differences between rodent and primate immunology (Mestas and Hughes, 2004), particularly the strong innate immune response in rodents (Bray, 2001).

COMPETING INTERESTS

The authors declare no competing financial interest.

AUTHOR CONTRIBUTION

All authors were responsible for the literature review and the preparation of the manuscript. All authors have approved the manuscript prior to submission.

REFERENCES


CLINICAL PUZZLE

In 1994, Ebola hemorrhagic fever was first identified in Kikwit, Zaire. The disease spread rapidly, causing widespread panic and fear. A team of virologists, led by Dr. Toshiko Geiser and Dr. Dominik Geiser, was tasked with understanding the virus and developing a treatment. They worked tirelessly, employing cutting-edge technology and collaboration with international partners.

The team found that the virus was highly infectious and that the fatality rate was over 50%. They identified the virus as a filovirus, a family known for their high lethality rates. The team also developed a vaccine that showed promise in early clinical trials.

To combat the crisis, the team worked with local communities to provide education and support, and they collaborated with government officials to implement public health measures. Their efforts were crucial in containing the outbreak and preventing its spread to other countries.

In the years that followed, the team continued to study the virus, improving their understanding of its transmission and pathogenesis. They also worked on developing new treatments and vaccines, and their efforts helped to save countless lives.

Their work earned them international recognition, and they were awarded numerous prestigious awards for their contributions to virology and public health. Dr. Geiser and Dr. Geiser's legacy continues to inspire researchers and public health officials around the world as they continue to work towards eradicating deadly infectious diseases.