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Edited by David A. Warrell, Timothy M. Cox, and John D. Firth

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Filoviruses

Chapter: Filoviruses
Author(s): J. ter Meulen
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Update:

Virus—Lloviu virus, new filovirus from bat Miniopterus schreibersii in Spain.

Pathogenesis—role of host genetics and cytokines.

Updated on 31 May 2012. The previous version of this content can be found **here**.

Essentials

Filoviruses are large RNA viruses, of which Ebola virus and Marburg virus cause the most severe forms of viral haemorrhagic fever and have been best-studied because of fear of their misuse as bioterrorism agents. These are zoonotic viruses with reservoirs, most likely fruit-eating bats, in the rainforests of tropical Africa, where they cause sporadic infections and outbreaks among great apes and humans.

Epidemiology—the primary mode of transmission of Ebola virus to humans often involves contact of hunters with dead animals, especially chimpanzees, whose meat is consumed as 'bush meat'; contact with bats has been implicated for Marburg virus. However, the viruses are highly infectious and are transmitted from the index case and subsequently from person to person by all body fluids, including sweat and respiratory droplets.

Clinical features—Ebola haemorrhagic fever is clinically indistinguishable from Marburg haemorrhagic fever. Presentation is with an influenza-like illness, often with gastrointestinal symptoms, followed by development of a maculopapular rash and haemorrhagic manifestations including epistaxis, gum bleeding, haematemesis, melaena, petechiae, and ecchymoses. There is no specific treatment, although recombinant activated protein C (Drotrecogin α) and the investigational anticoagulant rNAPc2 have reduced mortality by 20 to 30% in animal models. Mortality is 50 to 90%.

Diagnosis and prevention—viral haemorrhagic fever is a clinical diagnosis which requires the immediate instalment of the strictest barrier nursing procedures and notification of public health authorities. Care must be taken in both drawing and handling blood specimens, which must be inactivated before performing routine laboratory tests, and samples must be shipped immediately to a reference laboratory for diagnosis by detection of virus by cell culture, viral antigen by ELISA, and viral RNA by PCR. A prophylactic vaccine based on a replication-deficient adenoviral vector is in clinical development.

Introduction

Filoviruses are large, enveloped, negative-stranded, nonsegmented RNA viruses with a characteristic thread-like morphology, hence the family name *Filoviridae* (Latin *filum* = thread). Ebola viruses (EBOV), comprising three genetically distinct species from Côte d'Ivoire, the Democratic Republic of the Congo (DRC, formerly Zaire), and Sudan, and Marburg virus (MARV), cause the most severe forms of viral haemorrhagic fever (VHF). They are now among the best-studied agents of these diseases, mainly because of fear of their misuse as bioterrorism agents (Chapter 9.5.13). The first appearance of these viruses was in Marburg in 1967, when laboratory, medical, and animal care personnel exposed to tissues and blood from African Green monkeys (*Cercopithecus aethiops*) were infected. In 1976 and 1979, epidemics of a haemorrhagic disease with very high mortality in northern DRC (then Zaire) and in southern Sudan were found to be due to two strains of a related filoviruses, named Ebola virus. Over the next 10 years, rare, sporadic cases of filovirus infections in Africa were the only continuing evidence of the existence of these viruses. Another species of the virus, Ebola virus Reston (EBOv-R), was imported on four occasions between 1989 and 1996 with wild-caught monkeys (*Macaca fascicularis*) from Mindanao,

Republic of the Philippines, to animal facilities in the United States of America and Italy. This virus, which is highly lethal for monkeys, has caused asymptomatic infections in animal keepers. Since 1990, both Ebola and Marburg viruses have re-emerged across tropical Africa between latitudes 5° north and 5° south, causing several devastating epidemics.

In total, 18 instances of human Ebola haemorrhagic fever (EHF) have been recorded in Côte d'Ivoire, in the DRC, Gabon, Sudan, and northern Uganda. The outbreaks varied in size from 17 to 425 cases totalling 1880 cases, of which 1302 were fatal. The largest outbreak of Ebola virus disease so far (caused by EBOV Sudan) occurred in 2000 in Gulu, Uganda. There were 425 cases with a case fatality of 53%. Until 2007, Marburg virus cases totalled 567 with 467 fatalities. Outbreaks varied in size from 3 to 374, the largest in Uige, Angola where MARV appeared for the first time in 2005.

Aetiology, genetics, pathogenesis, and pathology

Filovirus infections are characterized by massive, unchecked, and destructive replication of virus in several organs, profound immunosuppression due to infection of immune cells and apoptosis of infected and noninfected cells, and triggering of a cascade of immune-mediated mechanisms resulting in a cytokine storm, endothelial damage, and coagulopathy culminating in shock and organ failure. The immunological and pathological aspects in endstage filoviral disease resemble, in several aspects, those of bacterial sepsis.

Through minute lesions in the skin and mucosa, the pantropic filoviruses infect initially dendritic cells, monocytes, and macrophages. Lymphocytes are spared from the infection. EBOV and MARV infected dendritic cells fail to mature to the antigen-presenting stage and do not produce proinflammatory cytokines required for activation of natural killer cells and T cells. At the molecular level, the expression of viral proteins interferes with the production of interferon-α (IFN-α) and β, and with the ability of these and IFN-γ to induce an antiviral state in cells. Dendritic cells show no increase in costimulatory molecules such as CD40, CD86, and interleukin 12 (IL-12). The early immune response dysfunction originating in dendritic cells is aggravated by continued replication of filoviruses in monocytes and macrophages, accompanied by the secretion of noninhibited proinflammatory cytokines and activation of polymorphonuclear leucocytes. This accumulated release of proinflammatory mediators culminates in a 'cytokine storm', causing thrombocytopenia and endothelial injury, e.g. through the action of tumor necrosis factor-α (TNFα). Fatal human Ebola cases showed a marked elevation of serum levels of IFN-γ, IL-2, and IL-10, whereas elevated IFN-α, TNFα, and IL-6 were associated with fatalities in some, but not all, studies. Increased blood levels of nitric oxide, which has been shown to contribute to hypotension, cardiodepression, and vascular hyporeactivity in sepsis, were also found to be associated with mortality. The likely reason for the variations of cytokine and chemokine release observed *in vivo*, as well as in experimentally infected primary human cells, is currently unknown genetic differences of the host. One study reported that HLA-B*07 and HLA-B*14 alleles were associated with survival, whereas HLA-B*67 and HLA-B*15 were associated with lethality in EBOV-infected patients. Both humans and experimentally infected nonhuman primates show massive apoptotic death of noninfected CD4+, CD8+, and NK cells in the blood and peripheral lymph nodes, a phenomenon which has been termed 'bystander apoptosis'. Lymphocyte apoptosis was thought to be responsible for an elimination of adaptive immune responses; however, studies in transgenic mice have not confirmed it as a major factor in the pathogenesis of disease. In addition, there appears to be also massive apoptotic death of infected macrophages.

The expression of tissue factor is up-regulated in infected monocytes and triggers the extrinsic pathway of coagulation. The procoagulant state amplifies the production of proinflammatory cytokines and the development of vascular leakage, which further provokes activation of coagulopathy. The terminal stage of the disease is therefore characterized by plasma leakage, disseminated intravascular coagulopathy, and bleeding. It is thought that triggering the above outlined cascade of events is more critical to the development of the observed pathology than direct organ damage due to cytopathic virus replication. However, infection of the liver and adrenal glands impairs the synthesis of clotting factors and steroids, thus aggravating hemorrhage and shock. Whether infection of endothelial cells contributes to the overall pathology remains controversial.

At autopsy, both Marburg and Ebola infected humans and primates show widespread haemorrhagic diathesis of skin, membranes, and soft tissue. Extensive necrosis with little infiltration is seen in parenchymal cells of many organs, including liver, spleen, kidneys, and gonads. The most characteristic histopathological features are seen in the liver. Large disseminated deposits of viral antigen can be found in different organs, including the sweat glands and the skin. Virus is also detectable in pneumocytes and as cell-free virions in the alveoli.

Spleen and lymph nodes show various degrees of lymphoid depletion with extensive vascular follicular necrosis. Fatal infection is marked by absence of specific IgG and presence of low levels of specific IgM in only 30% of cases, whereas in human survivors early and increasing levels of Ebola-specific IgM and IgG is followed by activation of cytotoxic T cells. During two outbreaks in Gabon, asymptomatic seroconversion with PCR-proven infection occurred in several people who mounted an early, strong but transient inflammatory response, with high levels of proinflammatory cytokines. This unexpected observation and data from animal models suggest that a tightly controlled, transient early type I IFN and pro-inflammatory cytokine response is able to induce protective antiviral innate and adaptive immune responses.

The recent successful immunization against EHF in animal models revealed that protection is clearly mediated by cellular immunity, because CD8+ T-cell depletion abrogated vaccine protection in nonhuman primates. Neutralizing antibodies are found neither in natural infection nor after immunization. However, antibodies may contribute to protection by non-neutralizing mechanisms.

Epidemiology

Central African nonhuman primates and monkeys are victims of EBOV, as are other animals such as bushpigs, porcupines, and antelopes living in the tropical rainforest. Data from wildlife surveillance show that epizootics occur more often than previously thought and that EBOV has caused massive die-offs of gorillas and chimpanzees. Phylogenetic analysis of the viruses further suggests that the outbreaks are epidemiologically linked and that EBOV, strain Zaire (EBOV-Z), has spread south-westward since 1976 in a wave-like manner from Yambuku, its site of appearance in the DRC, to the Republic of the Congo and to Gabon at a speed of approximately 50 km per year. This argues against the hypothesis that EBOV-Z was resident, but undetected, in the central African forest block before the mid 1970s. Evidence has now accumulated that fruit-eating bats (*Hypsignathus monstrosus, Epomops franqueti, Myonycteris torquata* and others) are one, but possibly not the primary, natural reservoir of EBOV, and hunting of bats for human consumption has been linked to an EBOV outbreak in DRC in 2007. Recently, EBOV Reston was detected in domestic swine in the Philippines and a few asymtomatic human infections were reported. The pathogenicity of the virus for these animals and their possible role in a transmission cycle are currently not known.

The primary mode of transmission of EBOV to humans often involves contact of hunters with dead animals, especially chimpanzees, whose meat is consumed as 'bush meat'. In several outbreaks, however, the mode of infection of the index case could not be elucidated. The index cases usually transmit the virus to caring family members, often women, who come into contact with blood and body fluids. These are highly infectious, so that the average rate of secondary cases generated from the index case is around 10 to 20%, but may be considerably higher. Occasionally, the virus has been spread through sexual contact. Nosocomial spread through improperly sterilized reusable syringes or other medical equipment has caused explosive Ebola epidemics in Sudan and the Democratic Republic of the Congo. The mortality among surgical staff operating on EHF patients misdiagnosed as having acute abdominal conditions was also extremely high. Nursing activities and preparing the corpse for burial carry a high risk of infection, as do burial practices which include touching of the corpse and collectively washing hands in a common bowel thereafter. There is no epidemiological evidence that Ebola or Marburg viruses are transmitted as true, small particle aerosols between humans. However, direct mucosal

exposure to droplets generated by a patient during coughing poses a considerable risk of infection.

MARV epidemiology is similar to that of EBOV. Evidence of infection has been detected in fruit-eating bats (*Rousettus aegyptiacus*) from Uganda and Kenya, and in insectivorous bats in DRC (*Miniopterus inflatus, Rhinolophus elocuens*). However, epizootics have not been observed in mammals. Contact with bats during mining activities was reported for several index cases of Marburg haemorrhagic fever (MHF), in accordance with cave roosting of *R. aegyptiacus*, a habit that is not observed in the bat species implicated in EBOV transmission. Until 2000, the viral origins of cases could be traced to eastern Africa. However, in 2005 the largest outbreak of MHF occurred in Uige, Angola, expanding the known range of the disease to the far western edge of the Congo basin. Continuing population movements in central Africa, destruction of the rainforest, and increased consumption of 'bush meat' increase the likelihood of future filovirus outbreaks. In 2008 a fatal and a nonfatal case of Marburg haemorrhagic fever occurred in the Netherlands and the United States of America, respectively, imported by tourists who had visited a bat-roosting cave in Uganda (Python cave, Queen Elizabeth park). Touching bat excrement or being hit by low-flying bats were identified as possible risk factors for acquisition of the infection.

Recently, a genetically distinct filovirus was discovered in Spain in dead insectivorous bats (*Miniopterus schreibersii*) and named Lloviu virus. There is currently no evidence of human infections with this virus.

Prevention

In endemic areas, avoidance of contact with bats and their excrements, with dead and diseased monkeys, and control of monkey sellers are currently the only feasible options for prevention. In case of outbreaks, interruption of person-to-person spread of the virus is essential for control. Early institution of safe and orderly care of the ill, using barrier nursing and disinfection procedures, should be set up with effective surveillance of high-risk contacts and prompt isolation of further cases (barrier nursing, guidelines from the CDC and WHO, see Chapter 7.5.17, Box 7.5.17.1). In fully equipped hospitals, patients must be placed in negative-pressure rooms and all personnel must wear protective gear with P3 filters for respiratory protection. Cutaneous or mucosal contact with blood or body fluids from an Ebola patient poses a high risk. Contacts must be followed up for development of persistent high fever for 3 weeks from the last date of contact by daily temperature measurement.

Development of vaccines against filoviruses has recently made astonishing progress, after decades of futile efforts. The first effective vaccine protocol against EBOV in nonhuman primates was based on a prime/boost regimen, expressing the viral nucleoprotein (NP) and glycoprotein (GP) from a plasmid (DNA immunization) and a recombinant, replication-deficient adenovirus, serotype 5 (Ad5). This vector has the advantage of having been tested extensively in humans and found to be safe. Subsequently, a protocol was developed in which a single shot of Ad5-GP given 4 weeks before challenge with 1000 infectious EBOV particles conferred 100% protection in nonhuman primates. This vaccine is currently in clinical trials performed by the National Institutes of Health, United States of America, and may be licensable within a few years. However, Ad5 vectors have the drawback of facing a high level of pre-existing neutralizing antibodies in the general population, which may impede the induction of anti-EBOV immunity. Prime/boost schemes will be required to overcome this problem. The latest amazing finding was that replication-competent vesicular stomatitis virus expressing the EBOV-GP could protect 50% of nonhuman primates when given 30 min after a lethal challenge, making it an ideal postexposure vaccine for health care workers. However, clinical development of this viral vector system faces higher regulatory hurdles, because it has so far not been evaluated in humans. Recently, an experimental preparation of the vaccine was given as a postexposure prophylaxis to a German researcher after a possibly EBOV contaminated needle-stick injury. No severe systemic side effects of the vaccination were reported.

Protection against MARV infection in animal models has been much easier to achieve using a variety of vaccines, including recombinant proteins, than against EBOV. This is probably due to the slightly slower replication of the virus in these models. A vaccine is likely to enter clinical trials soon.

Clinical features

MARV and EBOV cause identical clinical diseases. After an incubation period of 5 to 12 days, the disease starts suddenly with fever, headache, myalgia, and extreme fatigue. Early signs also include conjunctivitis, bradycardia, and sore throat, often associated with severe swelling and dysphagia, but no exudative pharyngitis. Severe nausea, vomiting, abdominal pain, and profuse watery diarrhoea are common. Around the fifth day, a perifollicular, nonitching, maculopapular rash frequently appears on the trunk, back, and shoulders, spreading to the face and limbs and becoming confluent (Fig. **7.5.18.1**). It may be difficult to see and has a measles-like appearance on dark skin. The rash fades in 3 to 10 days and is followed by a desquamation in survivors. In about half of the patients, haemorrhagic manifestations occur between the fifth and seventh day, including epistaxis, gum-bleeding, haematemesis (Fig. **7.5.18.2**), melaena, petechiae, ecchymoses (Fig. **7.5.18.3**), haemorrhages from needlesticks and post-mortem evidence of visceral haemorrhagic effusions. Dehydration and prostration are frequent; patients show the ghost-like facial expression typical of the disease. During the first week, the temperature remains high around 40°C, falling by lysis during the second week, to rise again between days 12 and 14. Other clinical signs during the second week include hepatosplenomegaly, oedema, orchitis, scrotal or labial reddening, myocarditis, and pancreatitis. Jaundice is not a feature. A poor prognosis is marked by haemorrhagic signs, oliguria or anuria, chest pain, shock, tachypnoea, and neurological symptoms (sudden hearing loss, blindness, painful paresthesia, intractable hiccups). Death in shock usually occurs 6 to 9 days after onset of clinical disease. Infection in pregnancy results in high maternal mortality and virtually 100% fetal death. Central nervous system involvement has led to hemiplegia and disorientation, and sometimes frank psychosis.

Fig. 7.5.18.1
Rash of Ebola haemorrhagic fever acquired through a laboratory accident.

(Courtesy of Professor D I H Simpson.)

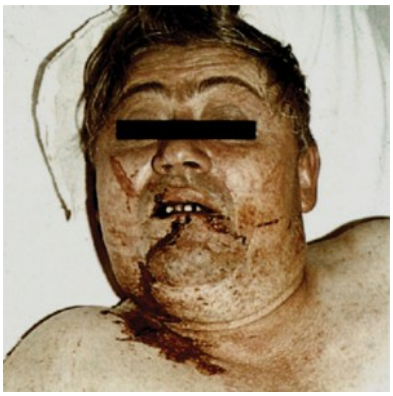

Fig. 7.5.18.2
Hemorrhage and oedema of face and neck in Marburg haemorrhagic fever.

(Courtesy: Professor S Stille.)

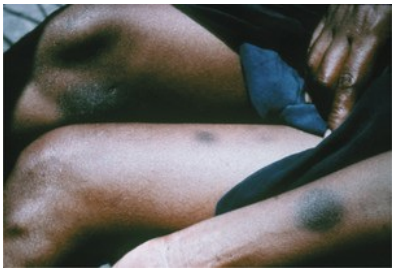

Fig. 7.5.18.3
Ecchymoses in a patient with Ebola haemorrhagic fever.

(Courtesy of Professor D I H Simpson.)

The recovery of Marburg and Ebola disease is prolonged with arthralgia or persistent arthritis, ocular disease (ocular pain, photophobia, hyperlacrimation, loss of visual acuity, uveitis), hearing loss and orchitis occurring as late manifestations. Serious but reversible personality changes have been recorded in a few survivors, namely confusion, anxiety, and aggressive behaviour. Blindness has been reported as a sequel.

Marburg virus has been isolated from the anterior chamber of the eye and from seminal fluid 7 weeks after the onset of clinical disease and there has been a documented case of sexual transmission. The shedding of EBOV RNA has been detectable in semen and vaginal fluid by polymerase chain reaction (PCR) for months, but not by virus isolation. Patients should therefore refrain from sexual activities during early reconvalescence.

Haematological studies reveal early leucopenia, thrombocytopenia accompanied by abnormal platelet aggregation, subsequent relative neutrophilia, and the appearance of atypical lymphocytes. Liver enzymes are elevated (AST/SGOT >ALT/SGPT) consistent with histopathological evidence of hepatitis (Fig. **7.5.18.4**), but alkaline phosphatase and bilirubin levels are usually normal or only slightly elevated. Although disseminated intravascular coagulation (DIC) is a prominent manifestation of EBOV infection in primates (prolonged prothrombin (PT) and partial thromboplastin time (PTT), D-dimers, fibrin split products), the presence of DIC in human filoviral infections has been a controversial topic, because logistical problems have hampered systematic studies in the past. However, fibrin deposition has been documented at autopsy, and clinical laboratory data suggest that DIC is likely to be also a prominent feature of human disease. In nonhuman primates, a rapid decline in plasma protein C levels was observed in EBOV infection, preceding clinical symptoms.

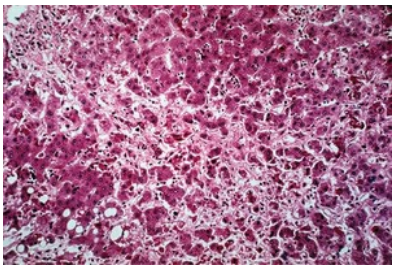

Fig. 7.5.18.4
Hepatic histology in Ebola haemorrhagic fever.

(Courtesy of Professor D I H Simpson.)

Differential diagnosis and criteria for diagnosis

Clinically, filovirus infections can be confused with nonviral infections such as severe malaria, typhoid fever, shigellosis ('diarrhée rouge' in francophone Africa), leptospirosis, rickettsial diseases, meningococcaemia, Gram-negative sepsis, and other conditions resulting in DIC. There is overlap of clinical presentation with other VHFs. Filovirus HF should be suspected in a patient living in or coming from, within the incubation period, a known endemic area (currently Angola, Côte d'Ivoire, the DRC, Gabon, Sudan, Kenya, and Uganda) and presenting with otherwise unexplained high fever (above 38.5°C) and vascular involvement (subnormal blood pressure, postural hypotension, petechiae, haemorrhagic diathesis, flushing of face and chest, nondependent oedema). Reported contact with another VHF patient or a known VHF vector is obviously a very important risk factor.

Because VHF is a purely clinical diagnosis which requires the immediate instalment of barrier nursing procedures and notification of public health authorities, rapid laboratory confirmation is mandatory. Care must be taken in both drawing and handling blood specimens since virus titre may be extremely high, and the virus is stable for long periods, even at room temperature. During the first week of clinical illness, virus is easily detected by cell culture, viral antigen by enzyme-linked immunoabsorbent assay (ELISA), and viral RNA by PCR, but all methods require specialized equipment. Blood samples have to be handled and shipped to a reference laboratory using special precautions (triple packaging: primary, secondary, and outer container with absorbent material in between) and have to be inactivated for performing routine laboratory tests (Chapter 7.5.17, Table 7.5.17.1). In fatal human EBOV cases, antiviral IgM and IgG antibodies were detected in 46% and 30% of patients respectively. ELISA or immunofluorescence can be performed, preferably in paired serum samples. A diagnostic test has been developed based on immunohistochemical detection of abundant filovirus antigen in biopsies. Skin snips taken from the axilla or nape of the neck are fixed with formalin and can be shipped without further safety requirements to reference laboratories.

For handling of clinical specimens from suspected cases, see Chapter 7.5.17, Table 7.5.17.1.

Treatment

Conceptually, therapy of EHF and MHF consists of specific antiviral approaches, modulation of the host immune response, and symptomatic treatment. Currently, no specific antiviral therapy is available. The guanosin analogue ribavirin is not effective against filoviruses. Prophylactic treatment of EBOV infection in nonhuman primates with high doses of either polyclonal immune serum, a potent neutralizing human monoclonal antibody (50 mg/kg), or IFN-α2b (2×10^7 IU/kg per day) delayed time to death but did not reduce mortality. However, transfer of convalescent whole blood to EBOV-infected patients protected 8/9 from lethal infection in an uncontrolled study, compared to 20% survival in untreated patients. Experimentally, modulation of the coagulation/inflammation cascade showed some promising results. Treatment with recombinant human activated protein C (continuous perfusion of 48 μg/kg per h drotrecogin-α, on days 0–7) resulted in 18.2% survival and a prolonged time-to-death. Similarly, treatment of nonhuman primates with the recombinant nematode anticoagulant protein c2 (rNAPc2), a potent inhibitor of FVIIa/tissue factor-initiated blood coagulation, by subcutaneous injections of 30 μg/kg bodyweight, administered once daily for up to 14 days after a high-dose lethal injection of Ebola virus, resulted in a 33% survival rate and prolonged survival time.

Fluid, electrolyte, respiratory, and osmotic imbalances should be managed carefully. Patients may require full intensive care support, including mechanical ventilation, along with blood, plasma, or platelet replacement. The maintenance of intravascular volume is a particular challenge, but every effort is justified since the crisis is short lived, and complete recovery can be expected in survivors. Treatment of all concurrent (tropical) infections is important.

Management of an imported EHF or MHF case will therefore require, to a certain degree, experimental therapy, such as the use of investigational drugs for modulation of immune responses and coagulation cascades, which are being evaluated in bacterial sepsis.

Prognosis

The case fatality of filovirus infections is extremely high and possibly dependent on the infecting species, with up to 90% for EBOV Zaire and MARV Angola. Because the lesions in filovirus infections are so widespread and the immune response is so ineffective, it is uncertain whether good supportive care alone has a major effect on the clinical outcome. Despite good clinical care being delivered to the majority of patients during the Ebola outbreak in Uganda in 2000, the overall mortality was not significantly lower than the 50% which would be expected for the Sudan strain of Ebola, which caused the epidemic. Common denominators of survival in filovirus-infected macaques are maintenance of D-dimer levels, maintenance of protein C activity (>50%), maintenance of levels of proinflammatory/procoagulant cytokines, and low viral load.

Areas of uncertainty/controversy

Despite concerted international actions, it has so far neither been possible to implement true standard of care patient treatment during filovirus outbreaks in Africa nor to conduct clinical trials. Therefore, most of the knowledge of the pathogenesis of these diseases and the few available therapeutic data come from experimental infection of nonhuman primates and uncontrolled clinical studies. While the importance of the type I IFN response in controlling filovirus infection is evident, it is unclear what constitutes a protective adaptive immune response either in natural infection or obtained through vaccination. Current data suggest that this may differ between different filoviruses and vaccine platforms.

Likely developments over next 5 to 10 years

The licensing of a recombinant, adenovirus-based EBOV and possibly MARV vaccine is to be expected in the United States of America within 5 years. Combined therapies using antiviral drugs, immune modulators, and anticoagulants will most likely improve survival rates in nonhuman primate models beyond the currently reported 20 to 30%.

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