EXPERT REVIEWS

Pathogenesis of arenavirus hemorrhagic fevers

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Viral hemorrhagic fevers (VHFs) caused by arenaviruses belong to the most devastating emerging human diseases and represent serious public health problems. Arenavirus VHFs in humans are acute diseases characterized by fever and, in severe cases, different degrees of hemorrhages associated with a shock syndrome in the terminal stage. Over the past years, much has been learned about the pathogenesis of arenaviruses at the cellular level, in particular their ability to subvert the host cell's innate antiviral defenses. Clinical studies and novel animal models have provided important new information about the interaction of hemorrhagic arenaviruses with the host's adaptive immune system, in particular virus-induced immunosuppression, and have provided the first hints towards an understanding of the terminal hemorrhagic shock syndrome. The scope of this article is to review our current knowledge on arenavirus VHF pathogenesis with an emphasis on recent developments.

KEYWORDS: arenavirus • hemorrhagic fever • hemorrhagic shock syndrome • immunosuppression • Junin virus • Lassa virus • vascular endothelium • viral pathogenesis

Arenavirus hemorrhagic fevers are devastating emerging human diseases

Viral hemorrhagic fevers (VHFs) are severe human diseases that show different clinical courses and are associated with the cardinal symptoms of fever, hemorrhages and shock. A wide variety of viruses that belong to different virus families can cause VHFs, including the filoviruses (Ebola and Marburg hemorrhagic fever [HF]), the arenaviruses (Lassa fever, Argentine HF [AHF], Bolivian HF, Venezuelan HF and Brazilian HF), bunyaviruses (Crimean Congo HF and Rift Valley fever) and flaviviruses (Yellow fever and Dengue HF) [1]. The arenaviruses are a large and diverse family of enveloped RNA viruses that include several causative agents of severe VHFs, which belong to the most devastating emerging human diseases and serious public health problems [1,2]. Arenavirus VHFs in humans are acute diseases characterized by fever and, in severe cases, different degrees of hemorrhages associated with a shock syndrome in the terminal stage. The most prevalent pathogen among the arenaviruses is Lassa virus (LASV), the causative agent of Lassa fever that is endemic to West Africa from Senegal to Cameroon and causes several hundred thousand infections per year with thousands of deaths [3]. The mortality of hospitalized Lassa fever patients is 15–30% and can reach more than 50% in some outbreaks. There is currently no vaccine available and therapeutic intervention is limited to intensive care and the use of ribavirin, which shows some efficacy when given early in disease. On the South American continent, the arenaviruses Junin virus (JUNV), Machupo virus (MACV), Guanarito virus (GTOV) and Sabia virus (SABV) have emerged as etiological agents of severe VHF in Argentina, Venezuela, Bolivia and Brazil, respectively [2]. The prototypic arenavirus lymphocytic chorio-meningitis virus (LCMV), which is distributed worldwide, is also a neglected human pathogen of clinical significance, especially in pediatric medicine [4,5] and represents a threat to immunocompromised individuals [6,7]. Considering the number of people affected and the unaddressed need for better therapeutics, arenaviruses arguably belong to the most neglected tropical pathogens. Apart from the severe humanitarian burden in endemic regions, arenavirus HF cases are regularly imported into metropolitan areas around the globe, placing local populations at risk [8]. This article will briefly cover some general aspects of the classification, epidemiology and basic virology of arenaviruses, and will then provide a more detailed overview of the clinical disease, and in particular the pathogenesis of arenavirus VHFs with an emphasis on recent developments in the field.

Classification & epidemiology of arenaviruses

The *Arenaviridae* are currently subdivided into two major subgroups, the Old World arenaviruses and the New World arenaviruses [9,10]. The Old World lineage contains the prototypic LCMV, the related Dandenong virus and the African viruses LASV, Mopeia, Mobala, Ippy and Morogoro virus. The New World arenaviruses are divided into three Clades, A, B and C. Clade A includes the South American viruses Pichinde virus, Parana virus, Pirital virus, Flexal virus and Allpahuayo virus. A North American Clade A/Rec comprises Whitewater Arroyo, Bear Canyon, and Tamiami, Catarina and Skinner Tank virus. The phylogenetic relationship of these viruses is poorly resolved, but there is evidence that a recombination event between Clade A and B viruses may be at the origin of this Clade as reviewed by Emonet *et al.* [9]. Whitewater Arroyo virus has been associated with three cases of fatal human disease [11,12]. However, a definitive causal relationship has not yet been established. Clade B contains the hemorrhagic viruses JUNV, MACV, GTOV, SABV and the recently emerged Chapare virus, as well as the nonpathogenic viruses Tacaribe (TACV), Amapari and Cupixi. Clade C contains the viruses Oliveros, Latino and Pampa.

In nature, each arenavirus species is carried by one or a limited number of related rodent species, which serve as their natural reservoirs. A possible exception is TACV, which has only been isolated from the fruit-eating bat species *Artibeus*. The present phylogenetic diversity of arenaviruses is the result of long-term co-evolution between viruses and their corresponding host species, involving vertical and horizontal transfer of viruses within and between populations, respectively, and probably occasional genetic recombination events [9,10,13].

A particular concern is the continued emergence of novel arenaviruses that are associated with fatal human disease. New arenaviruses emerge on average every 3 years [9]. In December 2003, a small cluster of fatal VHF cases was reported in a rural area near the Chapare River in Bolivia, Southern America. Examination of patient samples allowed the isolation and identification of Chapare virus, a novel species of arenavirus phylogenetically closely related to SABV [14]. More recently, in September 2008, a nosocomial outbreak of unexplained VHF occurred in Zambia, Southern Africa. Five patients were involved and four of them died with suspected human-to-human transmission. A high-throughput sequencing approach based on unbiased pyrosequencing revealed the presence of an arenavirus-like genome identified as a new member of the *Arenaviridae* family called LuJo virus according to its geographic distribution: Lusaka, Zambia and Johannesburg, South Africa [15]. The high mortality of four out of five hospitalized individuals and the strong evidence for human-to-human transmission are of particular concern regarding the epidemiological and disease potential of this new member of the arenavirus family.

Human arenavirus infections occur principally by aerosol or close contact with rodent excreta, contamination of food and drink or via skin abrasions. AHF caused by JUNV has a marked seasonal incidence, coinciding with the maize harvest between April and June when feral rodent populations reach their peak [16]. Agricultural workers and those in rural communities are thus at greatest risk. It is thought that transmission to humans results from the virus being carried on dust particles or by direct ingestion of contaminated foodstuffs.

Molecular & cell biology of arenaviruses

The basic virology of arenaviruses has been covered by excellent recent reviews [2,17] and only a short summary will be given here. Arenaviruses are enveloped viruses with a bisegmented negative strand RNA genome and a nonlytic life cycle restricted to the cytoplasm (Figure 1A). Each genomic RNA segment, L (circa 7.3 kb) and S (circa 3.5 kb), uses an ambisense coding strategy to direct the synthesis of two polypeptides in opposite orientation, separated by a noncoding intergenic region with a predicted hairpin structure (Figure 1B). The S RNA encodes the viral glycoprotein (GP) precursor, which is synthesized as a single polypeptide chain (circa 75 kDa) and post-translationally cleaved by the cellular proprotein convertase subtilisin kexin isozyme-1/site-1 protease to yield the mature virion GPs GP1 (40–46 kDa) and GP2 (35 kDa) [18–20], and the nucleoprotein (NP; circa 63 kDa). The L RNA encodes the viral RNA dependent RNA polymerase (RdRp or L polymerase; circa 200 kDa) and a small RING finger protein Z (circa 11 kDa).

Attachment to the host cell is mediated by the arenavirus GP1, which is located at the top of the mature GP spike present in the viral envelope. The transmembrane GP2 resembles the fusion-active membrane-proximal parts of other enveloped viruses. The cellular receptor for LASV, most other Old World arenaviruses and Clade C New World arenaviruses is α-dystroglycan (α-DG), a cell surface receptor for proteins of the extracellular matrix [21,22]. The hemorrhagic New World arenaviruses JUNV, MACV, GTOV and SABV can use human transferrin receptor 1 (TfR1) [23]. Upon attachment to the target cell, arenavirus particles are taken up by endocytosis and delivered to acidified endosomes where low pH induces membrane fusion [24]. New World arenaviruses like JUNV that use human TfR1 enter the cell via clathrin-mediated endocytosis [25]. In contrast, the Old World arenaviruses LASV and LCMV, which depend on α-DG, use a distinct and unusual pathway for cell entry that is independent of clathrin, caveolin, dynamin and actin [26–28]. Upon penetration into the cytoplasm, the viral ribonucleoparticles serves as a template for both transcription and replication that is mediated by the arenavirus RdRp L. The L and NP proteins are necessary and sufficient for these initial steps of viral transcription and replication [17]. Formation and release of arenavirus infectious progeny from infected cells occurs by budding during which ribonucleoparticles associate at the cell surface with membranes that are enriched in viral GPs. In arenaviruses, the Z protein functions as the main driving force for viral budding and resembles the matrix proteins of other enveloped RNA viruses [29,30].

Clinical disease & pathogenesis of Lassa fever

LASV was isolated in 1969 after a hospital outbreak in northern Nigeria [31,32], with additional outbreaks in Nigeria, Liberia and Sierra Leone. In 1972, the reservoir of LASV was identified as the rodent *Mastomys natalensis* [33]. Currently, LASV is
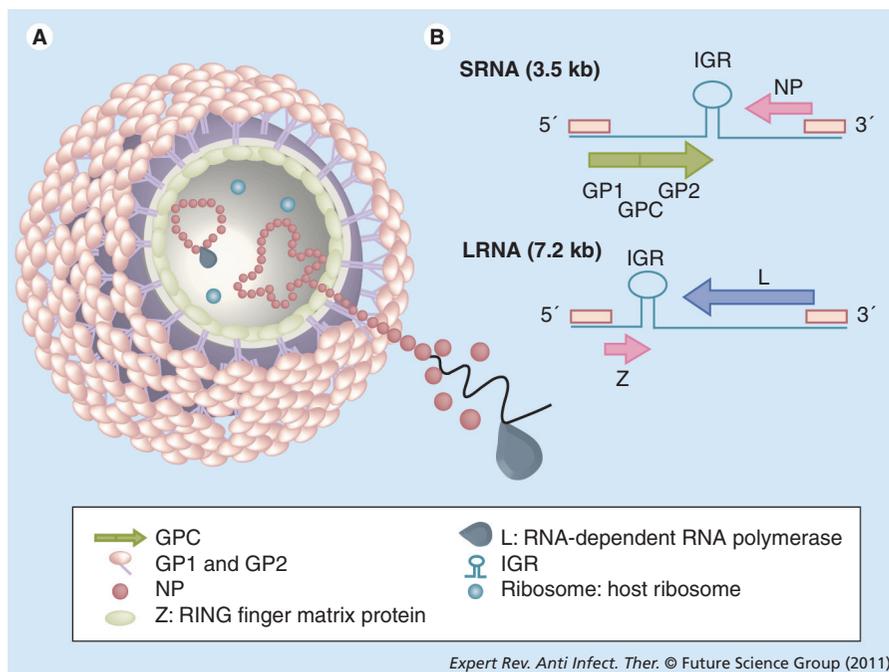
Figure 1. Arenavirus particle and genome organization. (A) Schematic representation of an arenavirus particle. The viral RNA is packaged into ribonucleoparticles (RNPs) containing the viral NP. The RNA-dependent RNA polymerase (L) is associated with RNP and is required for the initial steps of viral transcription. The matrix protein, Z, associates with the inner leaflet of the viral membrane envelope and interacts with the C-terminal part of the transmembrane GP2 moiety of the mature GP1/GP2 complex decorating the virion surface. The receptor-binding GP1 forms the top part of the GP virion spikes, whereas the transmembrane GP2 part contains the fusion machinery. In their mature form, arenavirus GPs are fully processed and presumably form trimers, similar to other fusion-active viral membrane proteins. **(B)** The ambisense coding strategy of arenaviruses. Each of the two ssRNA segments, L (circa 7.2 kb) and S (3.5 kb), uses an ambisense coding strategy to direct the synthesis of two polypeptides in opposite orientations and is separated by an IGR predicted to have a stable hairpin structure. The 5′ ends of the genomic arenavirus RNA cannot serve as a template for translation and viral protein expression requires prior transcription, as in true negative-strand viruses.
GP: Glycoprotein; GPC: Glycoprotein precursor; IGR: Intergenic region; L: RNA-dependent RNA polymerase; NP: Nucleoprotein.

endemic from Senegal to Cameroon [3] with recent emergence in Mali [34]. Reliable epidemiological data are missing from most affected regions, however, available information on seroprevalence for Nigeria, Sierra Leone and Guinea reveal that in some areas, 20–50% of the adult population have been infected with LASV [35], making LASV a major public health problem. Contact with urine or feces of persistently infected rodents invading human dwellings are the main routes of human transmission, and human-to-human spread can occur by contact with contaminated blood or body fluids [36,37]. The fatality rate of Lassa fever in hospitalized patients is between 15 and 20% [38], rising to more than 30% in pregnant women in the third trimester with fetal or neonatal loss of 88% [39]. The fatality ratio can be as high as 50% or greater in nosocomial outbreaks [37].

Acute Lassa fever presents with a wide spectrum of clinical manifestations from asymptomatic infection to fatal HF, and diagnosis based on early clinical symptoms is often difficult [38]. After an incubation period of 7–18 days, patients develop fever, weakness and general malaise. A majority of patients develop cough, severe headache and sore throat. Gastrointestinal manifestations such as nausea, diarrhea and vomiting are frequent. Signs of increased vascular permeability such as facial edema and pleural effusions occur in a minority of patients and indicate a poor prognosis. With severe cases leading to death, deterioration is rapid, occurring between the sixth and tenth day of illness with progressive signs and symptoms of pulmonary edema, respiratory distress, shock, signs of encephalopathy accompanied with seizures and coma, and bleeding from mucosal surfaces. Those recovering, generally 2–3 weeks after disease onset, clear the virus from the blood. A complication late in the course of disease or in early convalescence is sensorineural deafness [40].

A highly predictive factor for the outcome of LASV infection is the extent of viremia. Patients with fatal Lassa fever have higher viral loads at time of hospitalization and are unable to limit viral replication, whereas survivors have lower initial viral load and control the infection [41]. Despite the widespread infection and development of shock in terminal stages of the disease, histological examination of fatal Lassa fever cases showed surprisingly little cellular damage and only a modest infiltration of inflammatory cells [42]. Hepatic lesions in the form of multifocal hepatocellular necrosis were a consistent pathological change [42,43]; however, the degree of hepatic tissue damage was insufficient to cause hepatic failure and only minimal recruitment of inflammatory cells was observed. High viral titers were detected in the lung, spleen, kidney, heart and the adrenal gland [42]. Other histological alterations included interstitial pneumonia and acute myocarditis. In the spleen, necrosis occurred predominantly in the marginal zone of the splenic periarteriolar lymphocytic sheath.

A hallmark of fatal LASV infection in humans is the inability of the patient's innate and adaptive immune system to contain the virus, resulting in uncontrolled fatal infection. Instead of being recognized and contained by the host cell's innate defense system, pathogenic arenaviruses are able to subvert the normal mechanisms of innate pathogen recognition. The elucidation of the innate immune defense against arenaviruses and the mechanisms of viral countermeasures have been a particularly active area of arenavirus research in the past 5 years. As is the case for other RNA viruses, the genomic RNA of arenaviruses can be recognized by the cytoplasmic RNA helicases of the retinoic acid-inducible gene I (RIG-I) family, which represent the

major innate sensors for viral RNA and induce the production of type I interferons (IFNs) [44,45]. However, cells infected with arenaviruses are unable to induce a type I IFN response owing to the ability of the viral NP to act as an IFN antagonist [46,47]. Mechanistically, the NPs of arenaviruses block the activation of the transcription factor IFN regulatory factor 3 (IRF3), a major downstream regulator of type I IFN induction via RIG-I helicases [46]. The ability of arenavirus NP to block type I IFN induction is conserved among arenaviruses, with the exception of TACV, whose NP is much less efficient [48]. In contrast to Old World arenaviruses, where the NP appears to be the only IFN antagonist, the matrix protein Z derived from the New World HF viruses JUNV, MACV, GTOV and SABV is also able to perturb the activation of RIG-I [49]. The Z protein of New World HF viruses directly interacts with RIG-I and blocks the activation of downstream signaling involving IRF3 and NF-κB via the adaptor mitochondrial antiviral signaling protein. Interestingly, arenaviruses selectively prevent the induction of type I IFNs, but do not perturb signaling induced by the addition of exogenous type I IFNs [46]. The role of the type I IFN response in survivors of LASV infection is not clear. Recent studies in nonhuman primates revealed production of type I IFNs early in LASV infection of animals that survived [50], however, the source of IFN has not been clearly identified and it is at present not clear what role this IFN response plays in protection.

Fatal Lassa fever is characterized by a marked suppression of both branches of the adaptive immune response, cellular and humoral immunity. Surviving Lassa fever patients control the infection primarily by cellular immunity, in particular the antiviral T-cell response [51,52]. By contrast, antibodies play a modest role in acute LASV infection as patients can recover in the absence of a neutralizing antibody response [41]. The marked virus-induced immunosuppression in fatal Lassa fever likely involves infection of antigen-presenting cells (APCs), in particular macrophages and dendritic cells (DCs), crucial populations of professional APCs required to induce the adaptive antiviral immune response (FIGURE 2) [3]. Infection of human monocyte-derived macrophages and DC with LASV *in vitro* fails to activate the cells and results in an impairment of their ability to present antigens to T cells [53,54]. LASV infection of macrophages and DCs is not associated with the release of proinflammatory cytokines [53,54] and fatal Lassa fever in humans and nonhuman primates seems not to be associated with massive inflammatory and T-cell-derived cytokine release [50,55].

Another hallmark of LASV infection in humans and nonhuman primates is lymphopenia, which is most pronounced in fatal cases and associated with tissue damage in lymphoid organs [3]. Recent studies in nonhuman primates revealed that both CD4 and CD8 cells, as well as B cells and NK cells, were affected [50]. Since human T and B cells lack a functional LASV receptor [56,57], they are not infected, suggesting an indirect effect that may involve virus-induced immunoregulatory factors. Possible candidates are type I IFNs implicated in lymphopenia observed in experimental infection of the mouse with the related arenavirus LCMV [58].

Although hemorrhages represent a cardinal symptom of arenavirus VHFs, blood loss does normally not account for the fatal outcome of the disease. This is particularly true for Lassa fever, where bleeding is not a salient feature and does not significantly contribute to the shock syndrome [3]. LASV infection in humans is normally associated with only weak thrombocytopenia and mild perturbation of platelet function [59,60]. The platelet function defect appears to be mediated by a plasma inhibitor that has not yet been characterized [60]. Only mild vascular lesions were observed in postmortem examination of fatal human Lassa fever cases [42]. In contrast to other VHFs, disseminated intravascular coagulation involving activation of fibrin deposition is virtually absent in arenavirus VHFs (TABLE 1). Virus-induced impairment of vascular function is likely central to fatal Lassa fever (FIGURE 2) and perturbation of the function of endothelial cells precedes the onset of shock and death [59,61]. The mechanisms by which LASV infection affects endothelial cells are largely unknown and may include direct effects of the virus infection and indirect mechanisms, for example. virus-induced release of soluble factors that impact on endothelial cell function. In other VHFs, in particular Ebola and Marburg HF, alterations of vascular function have been attributed mainly to the virus-induced host responses, in particular excessive production of TNF-α, IFN-γ and nitric oxide, resembling some aspects of septic shock syndrome [62,63]. Initial *in vitro* studies infecting human monocytes/macrophages and human endothelial cells with LASV provided the first evidence for important differences between the pathogenesis of LASV and filoviruses. LASV infection was highly productive in these cell types without causing an overt cytopathic effect. In the absence of cell damage, LASV infection resulted in reduced levels of proinflammatory cytokines, including TNF-α and IL-8 [64]. Detection of proinflammatory cytokines in the sera of patients with fatal Lassa fever and experimental infection in nonhuman primates have so far revealed little evidence for a 'cytokine storm' as observed in filovirus VHF [50,55]. Although a possible role of host-derived soluble factors in LASV-induced perturbation of endothelial cell function *in vivo* can at present not be ruled out, it is conceivable that more direct effects of the virus on vascular endothelial cells as a consequence of viral gene expression may underlie at least some of the vascular dysfunction observed in fatal Lassa fever. In summary, the mechanisms underlying the profound shock associated with the terminal stage of fatal Lassa fever in humans are still largely unknown. According to a working model presented in FIGURE 2, infection of several cell types in different tissues, including the vascular endothelium, the liver and the adrenal cortex, may result in an overall impairment of the regulation of vascular permeability in a direct or indirect way. The terminal shock syndrome may involve an imbalance of fluid distribution between intravascular and interstitial spaces combined with coagulation abnormalities and perhaps dysregulation of blood pressure.

Clinical disease & pathogenesis of South American hemorrhagic fevers

Among the South American HF viruses, JUNV, the causative agent of AHF, represents the most important public health problem, whereas MACV, GTOV and SABV cause only sporadic

outbreaks. JUNV was isolated from fatal VHF cases in the humid Pampas, the major agricultural area of Argentina, in the 1950s [65,66]. The rodent *Calomys musculinus* serves as the main natural reservoir of the virus. The virus is occasionally isolated from other rodents, possibly because of transmission from the natural host population. Former endemic hot spots are currently cooling off, whereas the overall geographic regions affected by the disease increases progressively [67]. AHF is a severe illness with hemorrhagic and/or neurological manifestations and a fatality rate of 15–30% in untreated cases [16,67,68]. JUNV appears to be infectious as aerosols and deposits in the terminal respiratory bronchioles [69]. Upon early replication in the lung, the virus enters into the lymphoid system and spreads systemically. After an incubation period of 1–2 weeks, AHF starts with rather unspecific symptoms: fever, asthenia, muscular pain, dizziness, skin and mucosal rashes, and lymph node swelling. A total of 6–10 days after disease onset, symptoms worsen with cardiovascular, gastrointestinal, renal and neurological involvement, associated with

hematologic and hemostatic alterations. Disease manifestations are mainly neurological and/or hemorrhagic. Those who recover from AHF develop an antiviral immune response including antibodies in the second week of disease and clear the virus [70]. In convalescent plasma from AHF survivors, robust titers of neutralizing antiviral antibodies, mainly IgG, can be detected. In fatal cases, viremia is unchecked and patients succumb to a terminal hemorrhagic shock syndrome with a mortality of 15–30% in the absence of immune plasma therapy [16,68].

Pathological lesions in fatal AHF include generalized vasocongestion with multiple hemorrhages in the gastrointestinal mucosa, different organs, such as the liver, kidney and lungs, as well as subcutaneous tissue. In the kidneys, tubular and papillar necrosis is observed and scattered necrotic hepatocytes are found in the liver. No overt CNS pathology is apparent, despite the frequent manifestation of neurological symptoms. Secondary bacterial infections such as pneumonia are common in AHF. The lesions consistently associated with fatal cases are present
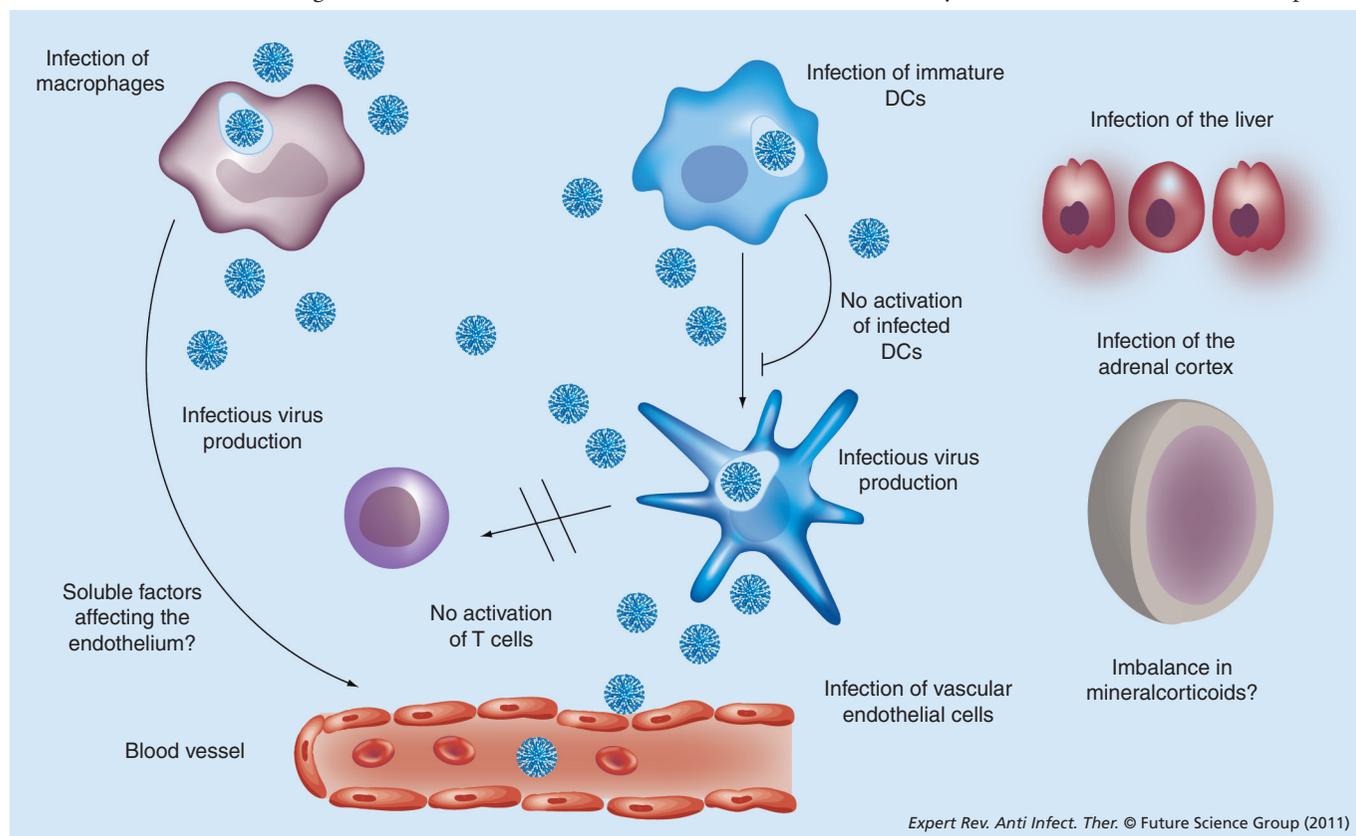

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Figure 2. Model for Lassa virus pathogenesis. Macrophages and DCs represent early and preferred targets for Lassa virus (LASV) in human infection. However, instead of being recognized and presented as foreign antigen, LASV establishes productive infection that fails to activate the cells and perturbs their ability to stimulate T cells, contributing to immunosuppression. LASV infection in macrophages and in particular DCs does not result in excessive production of cytokines, but releases significant amounts of infectious virus. Infection of the liver results in enhanced levels of transaminases and hepatocellular necrosis. While the histopathology in the liver is too weak to account for liver failure, infection of hepatocytes may alter the production of serum proteins, possibly affecting hemostasis. Extensive viral replication in the adrenal cortex, where high viral loads are detected, may affect the production of mineralcorticoids leading to fluid imbalance. LASV can productively infect vascular endothelial cells without causing overt cytopathology and vascular lesions are mild in fatal Lassa fever. The mechanisms underlying the alterations in endothelial cell functions that precede shock and death in fatal Lassa fever are largely unknown and may involve direct effect of virus infection as well as effects mediated by as yet undefined soluble factors released by other cells.
DC: Dendritic cell.

Table 1. Comparison of Lassa fever with the South American arenavirus hemorrhagic fevers and Ebola hemorrhagic fever.

Parameter	LASV	JUNV	MACV	Ebola
Fever	Yes	Yes	Yes	Yes
Hypotension	Yes	Yes	Yes	Yes
Hemorrhages	Weak[†]	Occasional	Occasional	Occasional
Lymphopenia	Yes	Yes	Yes	Yes
Hepatic lesions	Yes	Yes	Yes	Yes
Vascular lesions	No	No	No	No
Thrombocytopenia	Rare	Yes	Yes	Yes
Platelet dysfunction	Mild	Yes	Yes	Yes
DIC	No[‡]	Rare[§]	Rare[§]	Yes
Infection of MP	Yes	Yes	Yes	Yes
Infection of DC	Yes	Yes	Yes	Yes
Elevated interferons	No[¶]	Yes	Yes	Yes
Elevated cytokines	No[¶]	Yes	Yes	Yes

[†]Hemorrhages in LASV are limited to mucosal surfaces and blood loss is mild.
[‡]Fibrin deposits have been reported in rare cases.
[§]In isolated cases, there is biochemical evidence for DIC and detection of fibrin deposits.
[¶]No excessive levels of interferons and cytokines detected in Lassa fever patient sera.
DC: Dendritic cell; DIC: Disseminated intravascular coagulation; JUNV: Junin virus; LASV: Lassa virus; MACV: Machupo virus; MP: Macrophage.

in the lymphatic tissue with widespread necrosis of the splenic white pulp and in the cortical and paracortical areas of the lymph nodes. The bone marrow shows global cell depletion. The highest virus titers are found in the spleen, lymph nodes and lungs, and high levels of viral antigen are found in cells of the monocyte/macrophage lineage in peripheral blood [71], lymphatic tissue, lung and liver [72]. Patients with AHF have very high levels of IFNs, TNF-α, and other inflammatory mediators that correlate with the severity of disease [73–75]. However, at present it is unclear if the enhanced levels of cytokines reflect mainly enhanced levels of viral replication and more widespread infection or if they play a role in immune-mediated pathology similar to fatal filovirus VHFs [62,63].

Virus-induced immunosuppression is also a hallmark of fatal infection with the South American HF viruses. Similar to LASV, JUNV efficiently targets DC early in infection, likely contributing to immunosuppression (**Figure 2**). In contrast to Lassa fever, AHF manifests with more pronounced hemorrhages, although the extent of vascular damage and blood loss does not account for the fatal outcome [16]. The hemorrhagic syndrome in AHF is attributed to coagulation alterations and marked thrombocytopenia, possibly in combination with virus and cytokine-induced vascular damage and does, in contrast to other VHF, generally not involve disseminated intravascular coagulation (**Table 1**).

Several lines of evidence implicate infection of vascular endothelial cells in AHF pathogenesis. Infection of cultured endothelial cells with JUNV did not cause overt cytopathic effects but altered expression of cell adhesion molecules ICAM-1 and VCAM-1 and reduced secretion of coagulation factors, such as the prothrombic

von Willebrand factor (vWF) [76]. The latter finding seems to contradict clinical data showing increased vWF in sera of AHF patients [77] and the reason for this discrepancy is likely another source of vWF in AHF *in vivo*, such as megakaryocytes/platelets. Of particular interest is the increased production of the vasoactive mediator NO and prostaglandin PGI_2 in endothelial cells infected with a virulent strain of JUNV, but not an avirulent isolate [76], providing a first possible link between viral infection and the increased vascular permeability observed in fatal AHF cases.

A hallmark of fatal AHFs are coagulation abnormalities, including thrombocytopenia and low levels of platelet activity [78]. As in the case of Lassa fever, evidence for an inhibitor of platelet aggregation in plasma of infected patients has been found [60]. However, the exact nature of this inhibitor remains elusive. Recent studies shed light on the mechanism of virus-induced thrombocytopenia employing an *in vitro* model of human $CD43^+$ cells stimulated with thrombopoietin [79]. JUNV infection of $CD43^+$ precursor cells did not affect proliferation and cell viability but markedly perturbed the differentiation of megakaryocytes resulting in reduced thrombopoiesis at the levels of proplatelet formation and platelet release. Interestingly, perturbation of thrombopoiesis was not dependent on direct infection of cells, but seems to be, at least in part, mediated by a bystander effect that involves type I IFNs. A remarkable aspect of AHF is the fact that some patients die in absence of hemorrhagic disease and manifest mainly with neurological symptoms [16]. The pathophysiological basis for the neurological syndrome that is present to a variable degree in AHF patients is currently largely unknown.

MACV is also a rodent-borne pathogen that caused serious outbreaks of HF in Bolivia in the 1960s [80], but the number of cases has declined since. Human-to-human transmission has been reported [81]. GTOV emerged as the cause of Venezuelan HF in the 1990s [82,83]. Based on their close phylogenetic relationship to JUNV, infections with MACV [84,85] and GTOV [82,83,86] resemble AHF in their pathology, clinical manifestations and mortality.

While no specific treatments or vaccines have been established for MACV and GTOV, a live-attenuated JUNV has been developed and is used in high-risk groups in endemic areas [87]. Current therapy of AHF infection involves the administration of immune plasma from convalescent patients [88], which is effective when started during the first week of disease and critically depends on the titers of neutralizing antibodies. Immune plasma therapy reduces mortality from 15–30% to less than 1%. Circa 10% of patients develop a self-limiting late neurological syndrome, whose pathophysiology is unknown.

Insights into the pathogenesis of arenavirus hemorrhagic fevers from new animal models

The investigation of arenavirus pathogenesis critically depends on suitable animal models. Nonhuman primates are still considered the major relevant model for Lassa fever, in particular rhesus and cynomolgus monkeys (*Macaca mulatta* and *Macaca fascicularis*, respectively). Experimental studies in these nonhuman primates largely reproduce the pathology of human Lassa fever at the systemic and histological level, including hepatocellular necrosis, interstitial pneumonia, increased blood transaminase levels, and hemorrhagic signs and symptoms [50,51,89–94]. As in the human disease, the terminal shock syndrome includes hypovolemia, hypotension and acute respiratory distress. A recent study comparing antiviral immune responses between fatal and nonfatal LASV infection in cynomolgus monkeys revealed that fatal infection was associated with only weak activation of antiviral T cells and monocytes, a delayed and weak antibody response resulting in unchecked viremia, shock and death. Nonfatal infection was characterized by a vigorous antiviral T-cell response, activation of monocytes and effective control of viral replication [50]. The similarities with clinical findings in human Lassa fever underline the potential of this model for future studies. Marmosets have recently been described as an alternative nonhuman primate model for LASV infection [95]. Another powerful model for LASV infection in nonhuman primates uses the closely related LCMV, which is a BSL2/3 pathogen [96,97]. This model has recently been used for comprehensive transcriptome analysis in the blood and liver of rhesus monkeys infected with a lethal dose of LCMV that has provided an invaluable source for the search for candidate genes involved in arenavirus VHF pathogenesis and biomarkers for diagnostic purposes [98,99].

A major challenge in LASV research has been the lack of a mouse model for LASV that allows the application of useful mouse genetics for studies on LASV pathogenesis *in vivo*, mainly owing to the fact that normal laboratory mouse strains are resistant to LASV infection. A recent study found that mice expressing humanized MHC class I failed to control LASV infection and developed fatal VHF [100]. Interestingly, in this model, depletion of T cells prevented disease, irrespective of viremia, indicating a role for T cells in VHF pathogenesis. It will be of great interest to compare the observations from this novel small animal model with established nonhuman primate models and human infection. Another important lesson learned from this novel model is to consider the possibility of T-cell-mediated pathology in the development of novel Lassa fever vaccine strategies.

Other important BSL2/3 models for arenavirus VHF are the infection of Pichinde virus in guinea pigs (reviewed in [101]) and the infection of Syrian golden hamsters with Pirital virus [102,103]. Several experimental animal models exist for JUNV pathogenesis, including mice [104–107], guinea pigs [108–110] and rats [111], as well as New and Old World primates [112–116]. A particularly interesting small animal model for JUNV infection has recently been established using the JUNV Romero strain in guinea pigs and largely recapitulates the pathological findings in human AHF [117], holding great potential for basic research on JUNV pathogenesis and evaluation of novel drugs and vaccine candidates.

Expert commentary & five-year view

The past years have seen an impressive progress in our understanding of the basic molecular and cellular biology of arenaviruses. In particular, the advent of a powerful reverse genetic system for arenaviruses [118–120] paves the way for sophisticated structure–function studies and the molecular dissection of the mechanisms of virus–host interaction *in vitro* and also *in vivo*. Over the years, much has been learned about the pathogenesis of arenaviruses at the cellular level, in particular their ability to subvert the host cell's innate antiviral defenses [45–49]. Key IFN antagonists of arenaviruses have been discovered and the mechanisms by which the viruses block innate antiviral signaling elucidated. Other *in vitro* studies have shed light on the impact of arenavirus infection on the differentiation and function of cells targeted by hemorrhagic arenaviruses *in vivo*, including APCs such as macrophages and DCs [53,54], endothelial cells [76], and megakaryocytes [79] involved in platelet formation. At the same time, novel animal models provided important new information about the interaction of hemorrhagic arenaviruses with the host's adaptive immune system, in particular virus-induced immunosuppression, and provided the first hints towards an understanding of the terminal hemorrhagic shock syndrome [50,98–100,117]. However, many questions remain unanswered. In particular, the pathogenesis of Lassa fever appears to be rather atypical with little evidence for immunopathological mechanisms and largely unknown mechanisms underlying the vascular dysfunction involved in the terminal shock syndrome. As more subtle, direct effects of virus replication and gene expression may be responsible for the perturbation of endothelial cell function, future research involving suitable cell culture models for human endothelium, which allow detailed analysis of virus-induced cell biological and biochemical alterations, will be of great importance. Advances in endothelial cell culture, combined with high resolution confocal microscopy and the ability to measure endothelial cell functions such as transendothelial electrical resistance and hydraulic conductivity in live cells will allow monitoring of subtle, virus-induced functional changes as a consequence of arenavirus infection. The availability of a powerful reverse genetic system for arenaviruses [118–120] combined with suitable well-controlled *in vitro* experimental systems may allow the identification of viral proteins and their cellular targets implicated in virus-induced changes in the major cell types targeted by LASV *in vivo*, including APCs such as DCs and vascular endothelial cells. The identification of cellular proteins and signaling pathways affected by virus infection and the characterization of the molecular interactions involved over the next 5 years will open new avenues for the development of drugs that could prevent arenavirus-induced endothelial cell dysfunction associated with fatal arenavirus VHFs. In addition, recently developed novel diagnostic tools, including more sensitive molecular probes for viral nucleic acids and new antibodies against LASV proteins, will allow a better assessment of the extent of viral infection of the endothelium and consequent functional alterations *in vivo*. The recent development of novel and promising small animal models for human Lassa fever and AHF, as well as refinements

of established nonhuman primate models for LASV will greatly facilitate the validation of mechanistic findings obtained *in vitro* in the systemic context and allow evaluation of novel therapeutic strategies in preclinical studies.

Key issues

- Viral hemorrhagic fevers (VHFs) caused by arenaviruses are among the most devastating emerging human diseases and represent serious public health problems. There is no licensed vaccine against these pathogens and therapeutic options are limited.
- A particular concern with arenaviruses is the continued emergence of new viruses that are associated with severe VHFs, as illustrated by the emergence of Chapare virus in 2003 and Lujo virus in 2008.
- Arenavirus VHFs in humans are acute diseases characterized by fever and, in severe cases, different degrees of hemorrhages associated with a shock syndrome in the terminal stage. Blood loss in arenavirus VHF does not account for the fatal outcome, and the pathophysiology underlying the terminal shock syndrome is not yet fully understood.
- Based on its clinical and pathological features, Lassa fever presents as a rather atypical VHF. Postmortem examination of fatal Lassa fever cases show surprisingly little tissue damage and only modest inflammation with absence of disseminated intravascular coagulation in the terminal stage.
- Fatal arenavirus VHFs are characterized by marked immunosuppression of the host, resulting in uncontrolled viral spread and replication associated with hemorrhagic shock. The immunosuppression associated with fatal disease is likely related to the fact that hemorrhagic arenaviruses efficiently target antigen-presenting cells such as macrophages and, in particular, dendritic cells, and perturb their function in antigen presentation.
- Arenaviruses can efficiently block the host cell's antiviral interferon response with the viral nucleoprotein and, in some species, also the Z protein, acting as interferon antagonists. Efficient blocking of the host innate antiviral defense by arenaviruses may contribute to the inability of the host to contain virus infection in fatal arenavirus VHF.
- A number of novel animal models for VHFs have been developed in past years and hold much promise for future studies on arenavirus pathology *in vivo*.

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