The role of the vascular endothelium in arenavirus haemorrhagic fevers

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Summary
Viral haemorrhagic fevers (VHF) caused by arenaviruses are among the most devastating emerging human diseases. The most important pathogen among the arenaviruses is Lassa virus (LASV), the causative agent of Lassa fever that is endemic to West Africa. On the South American continent, the New World arenavirus Junin virus (JUNV), Machupo (MACV), Guanarito (GTOV), and Sabia virus (SABV) have emerged as causative agents of severe VHFs. Clinical and experimental studies on arenavirus VHF have revealed a crucial role of the endothelium in their pathogenesis. However, in contrast to other VHFs, haemorrhages are not a salient feature of Lassa fever and fatal cases do not show overt destruction of vascular tissue. The functional alteration of the vascular endothelium that precede shock and death in fatal Lassa fever may be due to more subtle direct or indirect effects of the virus on endothelial cells. Haemorrhagic disease manifestations and vascular involvement are more pronounced in the VHF caused by the South American haemorrhagic fever viruses. Recent studies on JUNV revealed perturbation of specific endothelial cell function, including expression of cell adhesion molecules, coagulation factors, and vasoactive mediators as a consequence of productive viral infection. These studies provided first possible links to some of the vascular abnormalities observed in patients; however, their relevance in vivo remains to be investigated.

Keywords
Endothelial cells, viral infection, virology

Introduction
Viral haemorrhagic fevers (VHF) are infectious diseases that show different clinical courses and are associated with the cardinal symptoms fever, haemorrhages, and shock. A wide variety of viruses that belong to different virus families can cause VHFs, including the filoviruses (Ebola and Marburg HF), the arenaviruses (Lassa fever, Argentine HF, Bolivian HF, Venezuelan HF, and Brazilian HF), bunyaviruses (Crimean Congo HF, Rift Valley fever), and flaviviruses (Yellow fever and Dengue HF). Arenaviruses VHF are among the most devastating emerging human diseases (1).

The most important pathogen among the arenaviruses is Lassa virus (LASV), the causative agent of Lassa fever that is endemic to West Africa from Senegal to Cameroon. On the South American continent, the New World arenavirus Junin virus (JUNV) causes a severe illness with haemorrhagic and neurological manifestations and Machupo (MACV), Guanarito (GTOV), and Sabia virus (SABV) have emerged as causative agents of severe haemorrhagic fevers in Bolivia, Venezuela, and Brazil, respectively (2). New arenaviruses emerge on the average one every three years as illustrated by the recent discoveries of Chapare virus and Lujo virus, newly emerged arenaviruses associated with fatal haemorrhagic fever cases in Bolivia and Southern Africa, respectively (3, 4). Apart from the severe humanitarian burden in endemic regions, arenavirus haemorrhagic fever cases are regularly imported into metropolitan areas around the globe placing local populations at risk (5).

Arenaviruses have a non-lytic life cycle and infection in mammalian cells is not associated with overt cytopathic effects (6, 7). In nature, each arenavirus species has as natural reservoir one or a limited number of closely related rodent species in which the virus is maintained by persistent infection transmitted in utero from the infected mother to the fetus. The present phylogenetic diversity of arenaviruses is likely 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 (8-12). In the natural host, persistent arenavirus infections are generally not associated with overt disease, despite high virus loads in most organs, including the central nervous system (6, 7).

Arenavirus VHF in man are acute diseases characterised by fever, and, in severe cases, different degrees of haemorrhages associated with a shock syndrome in the terminal stage. Clinical and experimental data indicate that the vascular system, in particular the vascular endothelium, is directly or indirectly involved in arenavirus VHF pathogenesis. While the pathogenesis of arenavirus VHF is similar in some aspects to other VHFs, e.g. caused by the filoviruses Ebola and Marburg virus, important differences exist, in particular in the case of Lassa fever that appears as a rather atypical VHF (Table 1). The present review will briefly cover some general aspects of the epidemiology, clinical disease, and pathology of arenavirus VHFs, and give an overview over clinical and experimental studies that have addressed the role of the vascular endothelium in arenavirus VHFs.

Epidemiology, clinical course, and pathogenesis of Lassa fever

LASV causes over 300,000 infections per year with several thousand deaths (13). However, these numbers are most likely underestimations as reliable epidemiological data are missing from most affected regions due to poor public health infrastructure and political and social turmoil in the past years. Indeed, available data for Nigeria, Sierra Leone, and Guinea reveal that in some areas, 20–50% of the adult population have been infected with LASV (14). 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, but has severe side effects in many patients. Considering the number of people affected, the human suffering involved, and the unaddressed need for better therapeutics, Lassa fever is arguably one of the most neglected tropical diseases. LASV was isolated in 1969 after a hospital outbreak in northern Nigeria (15, 16). In 1972, the reservoir of LASV was identified as the rodent Mastomys natalensis (17). Contact with urine or feces of persistently infected rodents is the main route of human transmission (18) and human-to-human spread can occur by contact with contaminated blood or body fluids (19-22). The fatality rate of Lassa fever in hospitalised patients is high, ranging between 15% and 20% (23).

Acute Lassa fever presents with a wide spectrum of clinical manifestations and differential diagnosis based on clinical symptoms is often difficult. The incubation period is 7–18 days, followed by 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 oedema and pleural effusions indicate a poor prognosis (23). With severe cases leading to death, deterioration is rapid with progressive signs and symptoms of pulmonary oedema, respiratory distress, shock, signs of encephalopathy accompanied with seizures and coma, and bleeding from mucosal surfaces (23). Those recovering, generally two to three weeks after disease onset, show disappearance of virus from the blood. A common complication late in the course of disease or in early convalescence is sensorineural deafness that can affect up to 15% of infected patients (24).

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 hospitalisation and are unable to limit viral spread, whereas survivors have lower viral loads that continue to be lowered progressively (25). The inability of the host to control viral infection in fatal Lassa fever cases is due to a marked virus-induced immunosuppression and patients die in absence of an adaptive anti-viral immune response (13). Despite the widespread viral replication and development of shock in terminal stages of the disease, histological examination of Lassa fever patients shows surprisingly little cellular damage and only a modest or negligible infiltration of inflammatory cells (26). Although hepatic lesions are a severe and consistent pathological change (27), the degree of hepatic tissue damage is insufficient to cause hepatic failure. In survivors, the host’s control of viral replication is primarily mediated by the anti-viral T-cell response.

Table 1: Viral haemorrhagic fevers (VHFs) caused by arenaviruses and filoviruses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LASV</th>
<th>JUNV</th>
<th>MACV</th>
<th>Ebola</th>
<th>Marburg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Hypotension</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Haemorrhages</td>
<td>weaka</td>
<td>occasional</td>
<td>occasional</td>
<td>occasional</td>
<td>occasional</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Hepatic lesions</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Vascular lesions</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>rare</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>DIC</td>
<td>nob</td>
<td>somec</td>
<td>somec</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Infection of MP</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Infection of DC</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

a Haemorrhages in LASV are limited to mucosal surfaces and blood loss is mild. b Fibrin deposits have been reported in rare cases. c In some cases biochemical evidence for DIC and detection of fibrin deposits.
and antibody responses appear to play little or limited role in acute infection (25).

As with other haemorrhagic arenaviruses, the severe acute diseases observed with LASV infection in humans, non-human primates, and some other species are in sharp contrast to the asymptomatic persistent infection in the natural rodent host, that maintains the virus in nature. Although the reasons for this are largely unknown, it is conceivable that co-evolution of arenaviruses with their rodent hosts over millions of years may have resulted in mutual adaptation of virus and host allowing an equilibrium between viral replication and innate host defense that limits viral replication to an extent compatible with normal host viability. In accidental hosts like humans, these control mechanisms may be lacking, allowing unchecked viral multiplication resulting in very high viral loads in different organs, consequent virus-induced perturbation of tissue function, and disease.

**The role of the vascular endothelium in Lassa fever pathology**

In contrast to other VHFs, haemorrhages are not a salient feature of Lassa fever and are largely limited to mucosal surfaces. When bleeding occurs in Lassa fever, it is associated with mild thrombocytopenia and perturbation of platelet function in vitro (28, 29) and blood loss is not thought to significantly contribute to the terminal shock syndrome. The platelet function defect appears to be mediated by a plasma inhibitor that has not yet been characterised (29).

As other arenaviruses, LASV has a non-lytic cell cycle and causes no overt cytopathic effect in vascular endothelial cells and other cell types (30). Hence, no specific vascular lesions were observed in post-mortem examination of fatal human cases of Lassa fever, and no vascular necrosis was observed in non-human primates experimentally infected with LASV (26). In contrast to other VHFs, including Ebola HF and Marburg HF, in fatal human Lassa fever, activation of fibrin deposition (disseminated intravascular coagulation, DIC) is rarely seen, suggesting a rather atypical pathogenesis of Lassa fever (Table 1).

Despite the lack of haemorrhagic disease manifestations, perturbation of vascular function is likely central to Lassa fever pathology as studies in non-human primates and human patients revealed endothelial cell function failure preceding the onset of shock and death (28, 31). Signs and symptoms of vascular dysfunction, such as facial oedema, likely due to increased microvascular permeability, are frequently associated with fatal disease outcome (23). The profound shock associated with the terminal stage of fatal Lassa in humans and primate models may result from overall impairment of the regulation of vascular permeability caused in a direct or indirect way by the virus on vascular endothelial cells. The consequent imbalance of fluid distribution between intravascular and interstitial spaces, together with some coagulation abnormalities and perhaps blood pressure dysregulation may cumulate in the observed lethal shock syndrome.

In contrast to filovirus VHFs, where a detailed model for vascular pathogenesis is currently emerging (32, 33), the mechanisms by which LASV affects endothelial cell function are largely unknown. Perturbation of endothelial cell function may include direct effects of the virus involving virus infection and gene expression and/or may occur in an indirect manner, by virus-induced release of soluble host-derived factors that affect endothelial cell function. During Ebola and Marburg virus infection, vascular alterations are thought to be mainly due to virus-induced host responses rather than a direct effect of the virus. Specifically, extensive infection of cells of the monocyte/macrophage lineage in filovirus VHF results in release of pro-inflammatory cytokines and vasoactive mediators, in particular tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and nitric oxide leading to a “cytokine storm”, resembling events observed in septic shock syndrome. Although cells of the monocyte/macrophage lineage represent also early targets of LASV, productive infection does not activate cells (34, 35) and detection of pro-inflammatory cytokines in sera of patients of fatal Lassa fever revealed no little evidence for a “cytokine storm” associated with lethal disease (36). Although these findings do not rule out a significant role of host-derived soluble factors in LASV-induced perturbation of endothelial cell function, more direct effects of the virus on vascular endothelial cells may underlie at least some of the vascular dysfunction observed in fatal Lassa fever.

Different subsets of vascular endothelial cells express the cellular receptor of Lassa virus, α-dystroglycan (37), and in vitro studies revealed efficient infection of human vascular endothelial cells by LASV (30). As in most other mammalian cell types, replication of LASV in endothelial cells, like e.g. human umbilical vein endothelial cells (HUVEC), was highly productive with virus titers of up to 10^7 PFU/ml without causing an overt cytopathic effect. In absence of cell damage, LASV infection in HUVEC resulted in perturbation of some cellular functions, as indicated by reduced levels of interleukin (IL)-8, a prototypic proinflammatory CXC chemokine (30). Interestingly, in contrast to LASV, infection of HUVEC with the related African arenavirus Mopeia, which has so far not been associated with haemorrhagic fever, had no effect on IL-8 production. The lack of IL-8 production in LASV infected endothelial cells correlates with the low levels of IL-8 and other pro-inflammatory cytokines in sera of at least some fatal Lassa fever cases (36). Currently, the extent of infection of vascular endothelial cells by LASV in vivo remains largely unknown, but it is conceivable that similar subtle changes in host cell function may occur and contribute to disease.

**Epidemiology, clinical course, and pathogenesis of the South American haemorrhagic fever viruses**

Among the South American haemorrhagic fever viruses, JUNV represents the largest threat for human health. JUNV was recognised as the causative agent of a VHF in the humid Pampas, the major agricultural area of Argentina, in the 1950s (38, 39). The rodent *Calomys musculinus* and several other rodent species represent the natural reservoir of the virus. The exact mechanism of rodent to human transmission is not known, but there is strong experimental evidence that these viruses are infectious as aero-
sols (40). While former endemic hot spots are currently cooling off, the disease area increases progressively, placing larger populations at risk (2).

Argentine haemorrhagic fever (AHF) is a severe illness with haemorrhagic and neurological manifestations and a case fatality of 15–30% (2, 41, 42). After an incubation period 1–2 weeks, AHF begins with fever and malaise, accompanied by mild neurological symptoms and early signs of vascular damage. Severe cases develop a frequently fatal haemorrhagic or neurologic disease. Those recovering from AHF improve during the second week of disease, develop a detectable antibody response after 12–17 days, and clear the virus (43).

After entry by inhalation (40), JUNV is able to spread into lymphoid organs, the endothelium, and the parenchyma of various organs. Lesions consistently found in fatal cases are present in the lymphatic tissue and bone marrow. Post-mortem, highest virus titers are detected in spleen, lymph nodes, and lung. The human disease is marked by immunosuppression, lymphopenia, and neutropenia. Severe secondary infections like pneumonia are common. High interferon levels and extensive activation of inflammatory mediators occur in acute AHF and correlate with fatal outcome (44, 45). Haemorrhagic manifestations are presumably due thrombocytopenia and haemostatic alterations but do generally not involve DIC (45, 46).

Machupo virus (MACV) is also a rodent-borne pathogen that caused serious outbreaks of HF in Bolivia in the 1960s (47, 48), but the number of cases has declined afterwards (49). Guanarito virus (GTOV) emerged as the cause of Venezuelan HF in the 1990s (50, 51). Recently, the disease incidence has significantly increased, posing larger populations at risk (52). Based on their close phylogenetic relation to JUNV, infections with MACV (47, 53, 54) and GTOV (50–52) resemble AHF in their pathology, clinical manifestations and mortality. A live attenuated JUNV vaccine has been developed and is used in high-risk groups in endemic areas (55). Current therapy of JUNV infection involves the administration of ribavirin and immune plasma from convalescent patients (56–58) that reduces mortality from 15–30% to under 1%.

The role of the vascular endothelium in the pathology of AHF

Regarding the involvement of the vascular endothelium in the pathology of the South American HF viruses, most work has focussed on JUNV and AHF. In contrast to Lassa fever, AHF manifests with more pronounced haemorrhages, although the extent of vascular damage seems also limited (42). AHF patient show significant thrombocytopenia, marked reduction in serum complement activity (43), and low levels of blood coagulation activity (45). As in the case of Lassa fever, evidence for an inhibitor of platelet aggregation in plasma of infected patients has been found (29). However, the exact nature of this inhibitor remains elusive. The cellular receptor of JUNV and the other South American HF viruses, transferrin receptor 1, is highly expressed on vascular endothelial cells (59) and high levels of productive infection are observed in vitro (60). Infection of cultured endothelial cells with virulent and non-virulent strains of JUNV did not cause cytopathic effects (61), in line with the absence of overt vascular tissue destruction in vivo (42). Productive infection of endothelial cells in culture with JUNV induced expression of ICAM-1 and, to a lesser extent, VCAM-1 (61). This up-regulation of cell adhesion molecules involved in endothelial cell activation strictly depended on viral replication as no effect was seen with UV-inactivated virus. In vitro, infection of endothelial cells with JUNV resulted in reduced expression and secretion of coagulation factors, such as the prothrombin von Willebrand factor (VWF). This finding seems to be in contrast to clinical data showing increased VWF in sera of AHF patients (46), suggesting another source of VWF during AHF in vivo. Interestingly, infection of endothelial cells with a virulent strain of JUNV, but not a non-virulent isolate markedly induced the production of the vasoactive mediator nitric oxide (NO) and prostaglandin PGI₂ (61), providing a possible link between viral infection to the increased vascular permeability observed in fatal AHF cases. These findings in vitro are clearly of great interest and studies on the role of endothelial cells in AHF in vivo using a suitable small animal model for AHF pathogenesis have a high priority. To this end, the establishment of a novel AHF model in guinea pigs using the Romero strain of JUNV that reflects many aspects of human AHF (62) holds promise for future research.

Conclusions and future perspectives

Clinical and experimental studies on arenavirus VHF have revealed a crucial role of the endothelium in the pathogenesis of these severe human diseases. However, while much has been learned in recent years about the role of the endothelium in the pathogenesis of the filoviruses Ebola and Marburg, much less is known in case the pathogenesis of arenavirus VHF. In particular Lassa fever appears as a rather atypical VHF, with little evidence for immunopathological mechanisms impacting on endothelial cell function. 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, are of great importance.

Advances in endothelial cell culture, combined with high resolution confocal microscopy and the ability to measure endothelial cell functions like transendothelial electrical resistance and hydraulic conductivity in live cells allow to monitor subtle, virus-induced functional changes as a consequence of arenavirus infection. Recent studies in the filovirus field used these powerful in vitro systems and provided interesting novel insights into the molecular and cellular mechanisms of virus-induced cellular alterations in endothelial cells that likely underlie the functional defects seen in vivo (32, 63). The use of recombinant viral proteins and recombinant viruses expressing individual proteins derived from haemorrhagic arenaviruses in these powerful in vitro experimental systems may allow the identification of viral proteins and their cellular targets implicated in virus-induced changes in endothelial cell function, as illustrated by recent studies on the effects of Ebola virus GP on endothelial cell activation and barrier cell function (64). The identification of cellular proteins and signaling pathways affected by virus infection...
Infection of the Endothelium

References


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and the characterisation of the molecular interactions involved may open new avenues for the development of drugs that could prevent arenavirus-induced endothelial cell dysfunction associated with fatal arenavirus VHF.

In addition, recently developed novel diagnostic tools, including more sensitive molecular probes for viral nucleic acids and new antibodies against LAV proteins, will allow a better assessment of the extent of viral infection of the endothelium and consequent functional alterations in vivo. In case of JUNV and AHF, recent in vitro studies revealed perturbation of endothelial cell function by the virus and provided a first possible link to some of the vascular abnormalities observed in patients. The advent of a novel and promising small animal model for human AHF, based on infection of guinea pigs with the Romero strain of JUNV, may allow the validation of these in vitro findings in the future.
