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Progress in the experimental therapy of severe arenaviral infections

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Summary

A number of viruses in the family *Arenaviridae* cause severe illness in humans. Lassa virus in West Africa and a number of agents in South America produce hemorrhagic fever (HF) in persons exposed to aerosolized excretions of the pathogens' rodent hosts. Because arenaviruses are not transmitted by arthropods, and person-to-person spread is rare, human infections occur singly and sporadically, and are usually not diagnosed until the patient is severely ill. Because the arenaviruses are naturally transmitted by the airborne route, they also pose a potential threat as aerosolized bioterror weapons. The broad-spectrum antiviral drug ribavirin was shown to reduce mortality from Lassa fever, and has been tested against Argentine HF, but it is not an approved treatment for either disease. Human immune convalescent plasma was proven to be effective for Argentine HF in a controlled trial. New treatments are needed to block viral replication without causing toxicity and to prevent the increased vascular permeability that is responsible for hypotension and shock. In this paper, we review current developments in the experimental therapy of severe arenaviral infections, focusing on drugs that have been tested in animal models, and provide a perspective on future research.

Keywords

arenavirus; viral hemorrhagic fever; Lassa fever; Argentine hemorrhagic fever; Machupo virus; Junín virus; Guanarito virus; Sabia virus; antiviral therapy

I. Arenaviral hemorrhagic fever

A. Introduction

The members of the family *Arenaviridae* are single-stranded, enveloped RNA viruses with an ambisense, bipartite genome. All of them are maintained in nature through chronic infection of rodents (or bats, in the case of Tacaribe virus). Several of them can cause disease in humans who come into contact with an infected animal or are exposed to its aerosolized excretions. The arenaviruses are traditionally divided into the Old and New World arenaviruses. The Old World agent, lymphocytic choriomeningitis virus (LCMV), is found worldwide as a rare cause of severe infection in infants and in immunocompromised individuals, including organ transplant recipients. In contrast, two other Old World agents, Lassa virus and Lujo virus, found in Africa, and five South American viruses (Junín,

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Machupo, Sabia, Guanarito and Chapare) cause a syndrome of hemorrhagic fever (HF) [1]. In contrast to the better-known Ebola HF, which begins abruptly and progresses rapidly to a severe systemic inflammatory syndrome with high levels of proinflammatory cytokines, coagulopathy and hemorrhage, arenaviral HF is characterized by a more insidious onset, the frequent presence of neurologic abnormalities, immunosuppression leading to high viremia, extensive organ damage, increased vascular permeability and shock [2]. Differences between Lassa fever and Argentine HF, the most common New World arenaviral disease, are noted below.

Because the arenaviruses are not carried by arthropods, and person-to-person transmission is rare, each arenaviral disease of humans is tightly tethered to the geographic range of its rodent reservoir host. All cases occur in rural areas, where people are most likely to come into contact with infected animals, either during agricultural work or in rodent-infested homes. Most infections are asymptomatic or mild, and do not reach medical attention. When a patient becomes sick enough to be hospitalized, the lack of rapid diagnostic methods means that a specific diagnosis is unlikely to be made until late in the disease course or during convalescence, through serologic testing. The inability of medical facilities in endemic areas to diagnose arenaviral HF in the early phase of illness, when treatment with antiviral drugs would be most effective, has discouraged efforts to develop effective therapies. Only in Argentina, where the presence of Junín virus in rodents poses a threat to large-scale agriculture, have researchers developed both a vaccine and a specific therapy and demonstrated their efficacy in controlled trials (see below) [3]. In contrast, no vaccine has been developed against the other types of South American arenaviral HFs or Lassa fever, and the only available countermeasure is the broad-spectrum antiviral drug ribavirin, which is not approved by the Food and Drug Administration for use against these diseases, and whose efficacy is largely unproven.

The fact that arenaviruses are naturally transmitted to humans in aerosolized rodent excretions indicates that these agents could be used as biological weapons, if propagated to high titer in cell culture and deliberately released as small-particle aerosols [4]. Lassa and the South American arenaviruses are therefore classified as Category A bioterror threats. Because of their airborne infectivity and the lack of vaccines and approved therapies, research on these agents is restricted to Biosafety Level (BSL)-4.

New therapies are needed to treat arenaviral HF in two different situations. For naturally occurring infections in endemic areas, which are usually diagnosed only after the patient has become severely ill, effective management will require a potent, nontoxic antiviral drug to block further viral replication, together with adjunctive therapies to prevent or reverse the increased vascular permeability that leads to multi-organ failure and death. For biodefense applications, in contrast, any new treatment must be usable in the setting of a large-scale bioterror attack, when large numbers of people have been exposed to an aerosolized virus, and others incorrectly believe that they have been infected. Any antiviral countermeasure should therefore be simple to deliver, preferably by the oral route, and safe enough to give to both infected and uninfected persons. In this paper, we review current efforts to develop new treatments that can be used both in arenavirus-endemic areas and for biodefense. We first briefly summarize the natural history of the Old and New World agents of arenaviral HF, and describe currently available therapies. We then review recent developments in antiviral drug development, highlighting those compounds that have shown efficacy in animal models and are most likely to be advanced to clinical trials.

B. Old World arenaviruses

1. Lassa virus (LASV)—The agent of Lassa fever (LF) is maintained in multimammate mice (*Mastomys natalensis*), which infest human habitations in rural areas of Nigeria, Sierra

Leone and other countries of West Africa. Serosurveys suggest that thousands of human infections occur each year. Most are mild or asymptomatic, but among patients who become sick enough to be hospitalized, the case fatality rate can reach 15-20% or higher. Poor infection control practices, such as the re-use of contaminated syringes, has resulted in outbreaks of LF in African hospitals, but person-to-person spread is otherwise rare [5]. Among more than 20 known instances in which people with incipient LF have travelled from West Africa to Europe or the United States, no secondary transmission has occurred [6]. Close contacts of LF patients are often treated with oral ribavirin, but there is no proof of benefit.

LF usually develops gradually, after a 1-3 week incubation period. Early signs and symptoms include fever, sore throat, headache and myalgias, which may progress to severe illness with vascular insufficiency and shock [2]. Although patients often show neurologic abnormalities on physical examination, viral invasion of the central nervous system has not been conclusively demonstrated. Hemorrhage may occur in severe cases. Patients with high levels of circulating virus or severe hepatic involvement, as shown by markedly elevated serum levels of alanine and aspartate aminotransferase, are at greatest risk of a fatal outcome [7].

Ribavirin has become an accepted therapy for LF, but its efficacy has only been assessed in a single clinical study, performed in Sierra Leone in 1986 [8]. When patients treated with the oral or intravenous (i.v.) drug were matched to past patients with similar levels of serum viremia or serum AST level, treatment was found to reduce the case fatality rate from roughly 50% to less than 20%. Ribavirin therapy was most effective if begun within 6 days after the onset of illness. Immune plasma, alone or in combination with ribavirin, has been shown to be protective in nonhuman primates infected with LASV, but limited clinical evaluation in patients in Sierra Leone showed less evidence of benefit [9, 10]. Efforts to develop new therapies for LF may be aided by the recent development of a model of LCMV infection in rhesus macaques, which shows typical features of HF [11]. Unlike LASV infection of macaques, LCMV may be studied in BSL-3 containment, offering a way for increased numbers of investigators to study arenaviral HF.

2. Other Old World viruses—In 2008, a novel arenavirus, now designated Lujo virus, was identified as the cause of severe HF in a patient in South Africa [12]. The nature of the illness was initially not recognized, permitting the spread of infection to 4 contacts. The last patient to become ill was the only one to receive ribavirin and to survive infection.

C. New World arenaviruses

1. Junín virus (JUNV)—Field mice in agricultural regions of central Argentina are the natural reservoir of JUNV, the agent of Argentine hemorrhagic fever (AHF). When the disease was first recognized in the 1950s, more than 1000 cases were diagnosed each year in farm workers. Researchers subsequently developed a live, attenuated vaccine (Candid #1), and its extensive use in endemic areas has reduced the incidence of AHF to a handful of cases each year [13].

Like LF, AHF often begins insidiously, with nonspecific signs and symptoms that include neurologic abnormalities. In contrast to LF, high levels of proinflammatory cytokines, coagulation abnormalities and bleeding are prominent features of disease. Most patients with a confirmed diagnosis become severely ill, and about one-third develop life-threatening hemorrhage or neurologic complications; however, there is no evidence of viral invasion of the central nervous system. The overall case fatality rate is approximately 15%. Treatment is based on the administration of immune plasma from AHF survivors. A placebo-controlled trial in the late 1970s showed that such therapy significantly reduced the case fatality rate,

providing it was initiated before the eighth day of illness [14]. The treatment regimen has since been standardized, based upon the titer of neutralizing antibodies in individual units of plasma. In contrast to untreated patients, who undergo a typical HF syndrome, patients who receive immune plasma sometimes relapse a few weeks after the completion of therapy with a variety of neurologic abnormalities. The etiology of this “late neurologic syndrome” is unknown [3]. It does not appear to result from viral replication in the central nervous system, and it usually resolves uneventfully. A small study of ribavirin therapy in AHF patients who had been ill for more than 8 days suggested that treatment was beneficial; a case of late neurologic syndrome was also observed [15].

2. Machupo virus (MACV)—In contrast to JUNV, which is maintained in mice that inhabit the margins of fields, MACV is found in rodents that infest homes in rural Bolivia. Human exposure therefore occurs in a manner similar to LASV. Severe hemorrhage is more common in Bolivian than Argentine HF, but the case fatality rate of the two diseases is roughly the same. However, even though no vaccine is in use, Bolivian HF is much less common than AHF, likely because the disease occurs in less populous areas and households can be kept largely free of rodents through simple control measures. Ribavirin is active against MACV infection in laboratory primates [16]. A few reports have described the treatment of patients with i.v. ribavirin, but no controlled studies have been performed [17]. In 2004, another highly pathogenic arenavirus, Chapare virus, was identified as the cause of a small cluster of fatal cases of HF in Bolivia [18].

3. Sabia virus (SABV)—Brazilian HF, caused by SABV, was first recognized in 1990, when a patient initially believed to have yellow fever developed a fatal hemorrhagic syndrome, and a virologist who investigated the case became ill, but survived [19]. Another laboratory-acquired infection with SABV occurred in the United States in 1994, when an investigator was exposed to the aerosolized agent through a centrifuge accident. His condition quickly improved with ribavirin therapy [20].

4. Guanarito virus (GTOV)—GTOV, the agent of Venezuelan HF, is maintained in cotton rats and cane mice in fields, so that male agricultural workers are at greatest risk of infection [21]. The clinical syndrome resembles AHF. More than 200 cases have been reported, with a case fatality rate of about 33%, but no specific therapy has been described.

II. Animal models of severe arenaviral disease

Clinical studies to evaluate new therapies for arenaviral HF in the regions where the diseases occur are hindered by the sporadic occurrence of human infections, the absence of rapid diagnostic tests and the lack of an advanced medical infrastructure. Moreover, because ribavirin has become an accepted therapy for LF, and immune globulin has been proven effective for AHF, a placebo-controlled trial of a novel antiviral therapy would probably be considered unethical. Animal models that accurately reflect the human disease are therefore essential for advancing promising therapeutic agents towards clinical use. A list of animal models available for the preclinical evaluation of candidate therapies is provided in Table 1.

Rodent models of arenaviral HF are most useful for early, proof-of-concept studies. Until recently, the lack of mouse models slowed the pace of research, but development efforts are now coming to the forefront. Recently, JUNV infection in AG129 mice, which lack cell-surface interferon (IFN)-alpha/beta and gamma receptors, was shown to be uniformly lethal, providing an alternative to studies in guinea pigs [22]. Like IFN receptor-deficient AG129 mice, animals lacking the *Stat1* gene also have a defective type I IFN response, rendering them susceptible to MACV infection [23], and suitable for antiviral studies. Although transgenic mice that express the human MHC-I have been shown to support LASV

replication and develop some signs of disease (22% mortality) [24], evaluation of antiviral interventions would be limited to assessing impact on viremia, viral replication in tissues and reduction of serum AST levels.

Antiviral testing in mouse models requires less housing space and permits the use of larger numbers of animals, resulting in greater statistical power, as well as the ability to perform multiple concurrent studies. Testing in mice also requires substantially smaller amounts of experimental compounds, which are often synthesized in limited quantities for the early stages of drug development. However, although the Machupo, Junín, and Lassa models will be useful, they are restricted by the requirement for BSL-4 containment, which is available in only a handful of laboratories. A more accessible model that can be worked with at BSL-2 is based on infection of AG129 mice with Tacaribe virus (TCRV) [25]. TCRV is closely related to JUNV and the other South American HF arenaviruses, and has been used to evaluate a novel therapeutic agent [25].

The recent increase in murine models of arenaviral HF should help spur antiviral drug and vaccine development, and provide proof-of-concept data for selection of the most promising lead compounds for further evaluation in guinea pigs and in nonhuman primates. These animal models of acute arenaviral disease, including those that employ surrogate viruses with minimal virulence for humans, have recently been reviewed [26], and are summarized in Table 1. The following sections review the activity of several promising drug candidates in a number of these models. Ultimately, drug testing in macaques, which are considered to provide the most accurate model of arenaviral HF of humans, will be critical for the advancement of experimental therapies under the US Food and Drug Administration's "Animal Rule."

III. New therapeutic approaches for arenaviral HF

Even though Argentine and Bolivian HF were first described in the late 1950s and Lassa fever in 1969, options for the specific therapy of these diseases remains limited to the broad-spectrum antiviral ribavirin and immune plasma. With the recent discovery of other New and Old World arenaviruses that produce similar syndromes, and the expectation that others will be discovered, it has become increasingly urgent to develop effective countermeasures. A substantial effort has therefore been made to identify substances with anti-arenavirus activity. This paper focuses on the most promising experimental therapies, which have shown protective activity in animals. These are listed in Table 2 and discussed in detail in the following sections, organized on the basis of their mechanism of action. Compounds that have demonstrated antiviral activity *in vitro*, but have not been evaluated *in vivo*, are listed in Table 3, but are not discussed further in this review.

A. Viral entry inhibitors

Several small molecules that inhibit the pH-induced endosomal membrane fusion that is crucial to arenavirus infectivity have recently been identified. The most promising agents, ST-193 (Fig 1A) and ST-294 (Fig 1B), disrupt the functional interaction between the G2 fusion subunit and the stable signal peptide by stabilizing the prefusion envelope glycoprotein (GPC) complex activation at low pH [27]. ST-294 was identified by high-throughput screening (HTS) for compounds active against TCRV replication in cell culture and shown to have *in vivo* activity in TCRV-infected newborn mice [28]. ST-193 was also identified by HTS, using a lentivirus pseudotyped with the LASV GPC, to discover small molecules that inhibit virus entry [29]. In contrast to ST-294, ST-193 inhibits both Old and New World arenaviruses. Genetic studies and competitive binding experiments suggest that both chemically distinct classes of inhibitors share a common binding pocket on the GPC, and that specificity is determined by inhibitor binding [27, 30]. Because of its broad-

spectrum activity, ST-193 appears to be the more favorable compound for further development. In a recent study, it was found to be efficacious in LASV-infected guinea pigs, providing treatment began prior to virus challenge [31]. Additional studies with ST-193 are needed to evaluate its therapeutic activity against both Old and New World arenaviruses in guinea pigs and nonhuman primates, either alone or in combination with ribavirin.

B. Nucleoside analogs

Favipiravir (T-705; Fig 1C) is a potent inhibitor of seasonal influenza viruses, and is also active against H5N1 avian and the pandemic H1N1 swine-origin strains, including viruses resistant to neuraminidase inhibitors [32-35]. It acts as a purine nucleoside analog, inhibiting the viral RNA-dependent RNA polymerase (RdRp) [36]. The orally active compound has advanced through Phase III clinical trials in Japan, and a new drug application (NDA) has been filed. Phase II trials are ongoing in the USA. In addition to its anti-influenza activity favipiravir inhibits a broad range of RNA viruses (reviewed in [37]). Approval for the treatment of influenza would facilitate its development for the treatment of other viral diseases, including arenaviral HF.

The anti-arenaviral activity of favipiravir was first described in studies of PICV in cell culture and in hamsters, in which it was protective in pre- and post-exposure prophylaxis studies [38]. In a subsequent study, the compound proved effective even when treatment was initiated nearly a week after virus challenge [39]. The therapeutic potential of favipiravir was further demonstrated in guinea pigs infected with a lethal dose of guinea pig-adapted PICV, in which complete protection was seen even when treatment was initiated after the animals had developed fever, weight loss, and thrombocytopenia, and substantial viral titers were present in plasma and tissues [40]. The inhibitory activity of favipiravir has also been confirmed against highly pathogenic strains of JUNV, MACV, and GTOV, with inhibition of the RdRp as the apparent mechanism of action [41]. Plans for future evaluation of favipiravir include the treatment of guinea pigs infected with a range of virulent arenaviruses (Table 1).

Fluorouracil (5-FU; Fig 1D) is a pyrimidine analog that has been in use for decades as a chemotherapeutic agent for cancer [42]. Several reports have described the extinction of LCMV by 5-FU treatment *in vitro*, through an increase in the mutation rate during genome replication [43-45]. However, the use of 5-FU as an *in vivo* antiviral therapy has been limited to mice lacking B and T lymphocytes that were chronically infected with LCMV [43]. Although the prevention of persistent infection was encouraging, evaluation of 5-FU in models of arenaviral HF is needed to further assess the potential of lethal mutagenesis as a therapeutic antiviral mechanism. The well-documented toxicity of 5-FU would make further development challenging.

Stampidine (Fig 1E), a nucleoside derivative of the retroviral reverse transcriptase inhibitor d4T, has been reported to be effective prophylactically in mice inoculated intracerebrally with LASV [46]. However, the study was confined to a single experiment, and no *in vitro* efficacy data were included. Presumably, the mechanism of action is through inhibition of the LASV RdRp. Stampidine's activity in a model of central nervous system infection suggests that it can cross the blood-brain-barrier, and might therefore help to prevent the neurologic disease that sometimes accompanies arenaviral HF [3, 47, 48]. Demonstrations of antiviral activity in cell culture and in more relevant models of arenaviral HF are needed to support further development.

C. Host targets

Host factors that play essential roles in viral replication are attractive targets for therapeutic intervention against severe viral diseases, because the approach reduces the problem of selection for drug-resistant viruses, and any associated toxicity may be tolerable during a short course of treatment. Several compounds that target host functions have been evaluated with some success in small animal models of arenaviral HF.

MY-24 (Fig 1F), an aristeromycin derivative believed to have activity as an inhibitor of S-adenosyl-L-homocysteine hydrolase, was found to have modest *in vitro* activity against TCRV, PICV and the Candid #1 strain of JUNV, prompting a series of *in vivo* studies in mice and hamsters [25]. Treatment consistently prevented death in mice challenged with TCRV, without reducing peak levels of virus in plasma or tissues. Although treated mice became ill, they did so several days after the control animals. Unfortunately, a similar degree of protection was not obtained in PICV-infected hamsters, in which treatment with MY-24 produced only a prolongation of the time to death and a reduction in liver disease [25]. Studies to further assess the antiviral activity and mechanism of action of MY-24 are ongoing.

Another compound with *in vivo* activity against arenaviral infections is genistein (Fig 1G), a plant-derived isoflavone with many biochemical activities, including that of a general tyrosine kinase inhibitor [49]. Following an initial demonstration of its *in vitro* activity against PICV [50], pre- or postexposure administration to hamsters resulted in significant protection against an otherwise lethal Pirital virus (PIRV) challenge [51]. Treatment also reduced virus titers in the serum and some tissues. *In vitro* studies suggest that genistein disrupts both entry and post-entry steps in arenavirus replication [52]. The compound also inhibits the entry of simian virus 40 polyomavirus [53]. Further studies in hamster or guinea pig models of arenaviral HF are needed to clarify its mechanism of action, toxicology, and pharmacokinetics, so as to define the most appropriate treatment regimens.

D. Immunomodulators

The type I IFN response plays a pivotal role in innate antiviral defense. Like other pathogens, the arenaviruses have evolved mechanisms to abrogate IFN responses at several points of activation [54-57]. The concept that successful defense against arenaviruses requires early and robust IFN responses is supported by studies of LASV-infected nonhuman primates [58] and by experiments evaluating recombinant IFN alfacon-1 (consensus IFN- α) in PICV-infected hamsters [59]. The demonstration of IFN efficacy prompted a follow-up study in which IFN alfacon-1 was combined with ribavirin [60]. Both additive and synergistic effects were observed when the combination was administered within 24 hours after virus challenge.

Despite these encouraging results, IFN- α therapy, alone or in combination with ribavirin, has not been pursued for the treatment of arenaviral HF, probably because of the high cost of producing type I IFN, the need for repeated doses and the systemic toxicity associated with bolus dosing. These problems can potentially be avoided through the delivery of a recombinant, replication-deficient type 5 human adenovirus (DEF201) encoding IFN alfacon-1, which elicits its production and release from infected cells. IFN alfacon-1 treatment was more effective when expressed from the DEF201 vector delivered intranasally compared to delivery by i.p. injection [59, 61]. It is hypothesized that the sustained, steady expression level and the native glycosylation pattern of host-synthesized IFN serve to enhance its antiviral effect. DEF201 is now being developed as a broad-spectrum platform for use against a number of viral pathogens, including the arenaviruses.

A novel approach that exploits the pathophysiology of viral infection has recently been described. Phosphatidylserine (PS) is a phospholipid component of the cell membrane that is normally maintained on the inner leaflet, but viral infection induces its exposure on the outer surface of the cell, effectively dampening the immune response both to infected cells and to viruses released from the cells [62-64]. Bavituximab is a chimeric monoclonal antibody that binds to circulating human plasma $\beta 2$ glycoprotein, and subsequently to PS on the surface of virus-infected cells and on enveloped viruses, promoting immune recognition and activation of antiviral defenses via antibody-dependent cell-mediated cytotoxicity. To date, bavituximab has been shown to have antiviral activity against PICV in guinea pigs, as evidenced by improved survival and by a reduction in viral titers in the serum and tissues [63]. It was also beneficial when given in combination with a sub-optimal dose of ribavirin. The evaluation of bavituximab in LASV or JUNV-infected guinea pigs, either alone or in combination with ribavirin or immune plasma, will be the true litmus test if this therapeutic platform is to move forward.

An overzealous inflammatory response that can trigger vascular leakage, hypotension and shock is believed to underlie the pathogenesis of many of the viral HFs [1, 65]. Controlling systemic inflammation may therefore limit disease severity. In one approach to limiting the extent of inflammation, HTS was used to identify small molecules that blocked the toll-like receptor 2 (TLR2)-mediated response to LCMV infection [66]. Of these, compound E567 (Fig 1H) was selected for further investigation. In a number of *in vitro* experiments, E567 abrogated LCMV-induced inflammatory responses in primary mouse macrophages and in primary human monocytes. E567 pre-treatment also significantly reduced viral titers in the spleens of LCMV-infected mice and the peak serum level of the proinflammatory chemokine MCP-1 [66]. The weakened inflammatory response may be due to the independent or combined effects of E567 inhibition of TLR signaling and/or viral replication. Further studies in rodent models of arenaviral HF are needed to gain a better perspective on the effectiveness of this and other compounds that act by modulating TLR2-directed inflammation.

E. Modulation of host responses

Because the pathophysiology of viral HF includes stimulation of procoagulant pathways and increased permeability of the vascular endothelium, these processes are being evaluated as possible targets for therapeutic intervention. Recombinant nematode anticoagulant protein c2 (rNAPc2) is a long-acting anticoagulant that blocks initiation of the extrinsic coagulation pathway by inhibiting the tissue factor-factor VIIa complex [67, 68]. rNAPc2 proved highly protective in macaques infected with an otherwise uniformly lethal dose of Ebola Zaire virus, when initiated the day following virus challenge [69]. In addition to reducing coagulation abnormalities, treatment dampened inflammatory responses and significantly lowered peak circulating virus titers. A similar approach was somewhat less effective in animals infected with the highly virulent Angola strain of Marburg virus [70]. Given the somewhat different pathophysiology of arenaviral HF, it is not known whether rNAPc2 would be similarly beneficial for these diseases. Should it prove to be beneficial in animal models of arenaviral HF, it might be given together with drugs that target viral replication.

Prevention of the “vascular leak” that is responsible for hypotension and shock in viral HF is also being evaluated as a therapeutic strategy. Increased endothelial permeability is stimulated by circulating factors such as TNF- α , interleukins, and thrombin, which trigger signaling cascades that disrupt intercellular VE-cadherin interactions, or may potentially result from viral infection of the endothelium or other direct virus-mediated effects. Recent studies have identified an endothelial cell-surface receptor, Robo4, which when activated by its endogenous ligand, Slit protein, preserves the integrity of the endothelial barrier by promoting VE-cadherin binding [71]. Treatment of mice with recombinant Slit protein

prevented death in a standard sepsis model and was protective in a uniformly lethal model of H5N1 avian influenza, providing proof of concept for therapies that aim to protect the host against the deleterious effects of its own immune mediators [72]. We have shown that vascular leak occurs in arenaviral HF in rodents, and that its timing and extent can be measured through simple assays [73]. We plan to use this model to evaluate combination therapies, in which drugs such as ribavirin are used to inhibit viral replication, while adjunctive treatments such as rNAPc2 or the Slit protein prevent damaging host responses.

IV. Future perspective

Arenaviral infections are a challenging target for antiviral drug development. Because these diseases occur sporadically in rural areas of Africa and South America, where humans come into contact with chronically infected rodents, cases are uncommon and are rarely identified before the patient is severely ill. Fortunately, the last 50 years have shown no evidence that the arenaviruses are capable of extended person-to-person transmission, and there is no reason to believe that expanding epidemics will occur. In most regions where arenaviral HF occurs, human disease can be largely prevented through rodent control measures. Success in reducing the incidence of Argentine HF through targeted use of the Candid #1 vaccine shows that vaccination is a cost-effective strategy in areas of high rodent-human contact.

The situation is different in terms of the potential threat of arenaviruses as bioterror agents. Because they are stable when released as aerosols, Lassa virus or the South American agents could be propagated and used as weapons against population centers. A deliberate release would result in a wave of severe illness in persons exposed to the aerosol, but further person-to-person transmission would be minimal. As for the naturally occurring disease, methods of diagnosing arenaviral HF early in the course of illness will be critical for ensuring the success of therapy.

Given the small number of naturally occurring cases and the difficulty of predicting the pathogens most likely to be used by bioterrorists, the best strategy for countering the arenavirus threat is to develop a safe, easily administered, broad-spectrum antiviral drug that will be active against both Old World and New World viruses. Because many patients will only be diagnosed after severe illness has developed, a countermeasure is also needed to mitigate the damaging effects of host inflammatory responses, especially the increased vascular permeability that is responsible for multi-organ failure and shock. Progress towards the latter goal will require the refinement of animal models, including improved methods of measuring changes in vascular permeability during the course of illness.

V. Executive summary

A comprehensive strategy to reduce the impact of severe arenaviral infections in endemic areas will incorporate the following elements:

- rodent control measures, to reduce human exposure to infected animals;
- vaccines to protect those at greatest risk of infection;
- rapid diagnostic methods, to identify infected individuals early in the course of illness;
- a broad-spectrum antiviral drug, that ideally is effective both against arenaviruses and other viruses that more commonly infect humans;
- adjunctive therapies to block the increased vascular permeability that is the proximate cause of death in arenaviral HF.

Points 3-5 apply equally to the development of defenses against arenaviruses used as bioterror weapons.

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Figure 1.
Chemical structures of compounds evaluated in small animal models of arenavirus infection.

Table 1

Animal models for the study of arenaviral HF

Virus	Disease	Animal model	Selected references
Lassa	Lassa fever	Rhesus macaque	[74], [75], [76]
		Cynomolgus macaque	[77], [78], [79]
		Marmoset	[80]
		Guinea pig	[81], [82]
	Lassa virus replication	HHD ^a mouse	[24]
		MHC-I ^{-/-b} mouse	[24]
LCM	Lassa fever	Rhesus macaque	[83]
Pichindé		Guinea pig ^c	[84], [85], [86]
		Hamster	[87], [88], [73]
Piritral		Hamster	[89], [90]
Junín	Argentine hemorrhagic fever	Rhesus macaque	[91], [92]
		Marmoset	[93], [94]
		Guinea pig	[95], [96], [97]
		AG129 ^d mouse	[22]
Tacaribe		AG129 ^d mouse	[25]
Machupo	Bolivian hemorrhagic fever	Rhesus macaque	[98], [99], [100]
		Cynomolgus macaque	[99]
		African green monkey	[101], [102]
		Guinea pig	[103]
		STAT-1 ^{-/-e} mouse	[23]
Guanarito	Venezuelan hemorrhagic fever	Guinea pig	[104]

^aGenetically engineered mouse that expresses a human/mouse-chimeric HLA-A2.1 molecule in place of the murine MHC-I.

^bβ2-microglobulin-deficient mouse (MHC-I^{-/-}).

^cVirus adapted to produce lethal disease.

^dType I and II interferon receptor-deficient mouse.

^eSignal transducer and activator of transcription 1-deficient mouse (STAT-1^{-/-}).

Lymphocytic choriomeningitis, LCM; aspartate aminotransferase, AST.

Table 2

Compounds active in animal models of arenaviral HF

Compound	Known/suspected drug target	Structure	Infection model(s)	Selected references
ST-193	Fusion	Small molecule	LASV guinea pig	[31]
ST-294	Fusion	Small molecule	TCRV mouse ^a	[28]
Favipiravir (T-705)	Viral polymerase	Pyrazine derivative	PICV guinea pig, PICV hamster	[38-40]
Stampidine	Viral polymerase	Nucleoside analog	LASV mouse ^b	[46]
5-Fluorouracil	Mutagen	Base analog	LCMV mouse ^c	[43]
Genistein	General kinase inhibitor	Isoflavone	PIRV hamster	[51]
MY-24	SAH hydrolase	Aristermoycin derivative	TCRV mouse	[25]
Bavituximab	Phosphatidylserine	Monoclonal antibody	PICV guinea pig	[63]
E567	TLR2 signaling	Small molecule	LCMV mouse ^d	[66]
DEF201	IFN pathway	Adenovirus-vectored IFN	PICV hamster	[61]
Infergen	IFN pathway	Consensus IFN	PICV hamster	[59, 60]

^aTCRV infection of newborn mice^bCBA mice challenged by intracerebral injection of LASV^cLCMV persistent infection in B- and T-cell-deficient mice^dLCMV-Armstrong infection of mice

S-adenosyl-L-homocysteine, SAH.

Table 3Compounds with *in vitro* anti-arenavirus activity

Compound(s)	Known/putative target	Structure	Selected references
Amphipathic DNA polymers	Virus-host cell receptor interface	Phosphorothioate oligonucleotides	[105]
8C1, 17C8, and other derivatives	Fusion	Small molecules	[106]
F100G5	Fusion	Monoclonal antibody	[107]
Chlorophyllide and derivatives	Viral envelope	Alkylated porphyrins	[108]
LJ001	Viral lipid membrane	Small molecule	[109]
N and L siRNA	N and L proteins	siRNA	[110]
NSC14560	RING finger motif of Z protein	Thiuram disulfide	[111]
NSC20625	RING finger motif of Z protein	Aromatic disulfide	[112-114]
Z2-siRNA	Z protein	siRNA	[115]
Decanoyl-RRLL-chloromethylketone	Cellular site 1 protease (S1P)	Peptide-derived small molecule	[116, 117]
PF-429242	Cellular site 1 protease (S1P)	Small molecule	[118]
2OHM, 13OM	N-myristoyltransferase (NMT)	Myristic acid analogs	[119]
Alpha and gamma IFNs	Interferon (IFN) pathways	IFNs	[120]
Azoles and derivatives	Undefined	Small molecules	[121-123]
Brassinosteroids	Undefined	Ethylbrassinone analogs	[124]
DHEA, EA, and derivatives	Undefined	Steroids	[125]
N-substituted acridones	Undefined	Acridones	[126]
Tetralonethiosemicarbazone	Undefined	Small molecule	[127]

Dehydroepiandrosterone, DHEA; epiandrosterone, EA; DL-2-hydroxymyristic acid, 2OHM; 13-oxamyristic acid, 13OM.