Chapter 21

HEMORRHAGIC FEVER-CAUSING MAMMARENAVIRUSES

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INTRODUCTION

The family *Arenaviridae* includes the two genera *Mammarenavirus* and *Reptarenavirus*, which are established to accommodate mammalian and reptilian arenaviruses, respectively. Seven mammarenaviruses cause viral hemorrhagic fever in humans: Lassa virus (LASV), Lujo virus (LUJV), Chapare virus (CHAPV), Guanarito virus (GTOV), Junín virus (JUNV), Machupo virus (MACV), and Sabiá virus (SABV). The clinical course and pathology of the viral hemorrhagic fevers caused by these viruses can differ, and therefore various diagnosis and treatment options are available. This chapter summarizes similarities and disparities between the viruses and the diseases they cause.

HISTORY AND EPIDEMIOLOGY

Old World Mammarenaviruses

Lassa fever is a severe disease common in areas of western sub-Saharan Africa (Nigeria, Liberia, Guinea, Sierra Leone; Figure 21-1). Lassa fever was first described in Jos, Nigeria, in 1969 in a hospitalized patient and a caretaker, both of whom became severely ill and subsequently died.[1] The etiologic agent of Lassa fever is LASV. The case fatality rate of Lassa fever is about 1% to 2% in the endemic areas, with an estimated 300,000 to 500,000 infections annually. The disease is especially severe late in pregnancy.[2] Infections tend to be more common in February to April compared to the rest of the year.

In September and October of 2008, LUJV was discovered during a nosocomial viral hemorrhagic fever outbreak. The index case became infected in Lusaka, Zambia (Figure 21-1), but the origin of

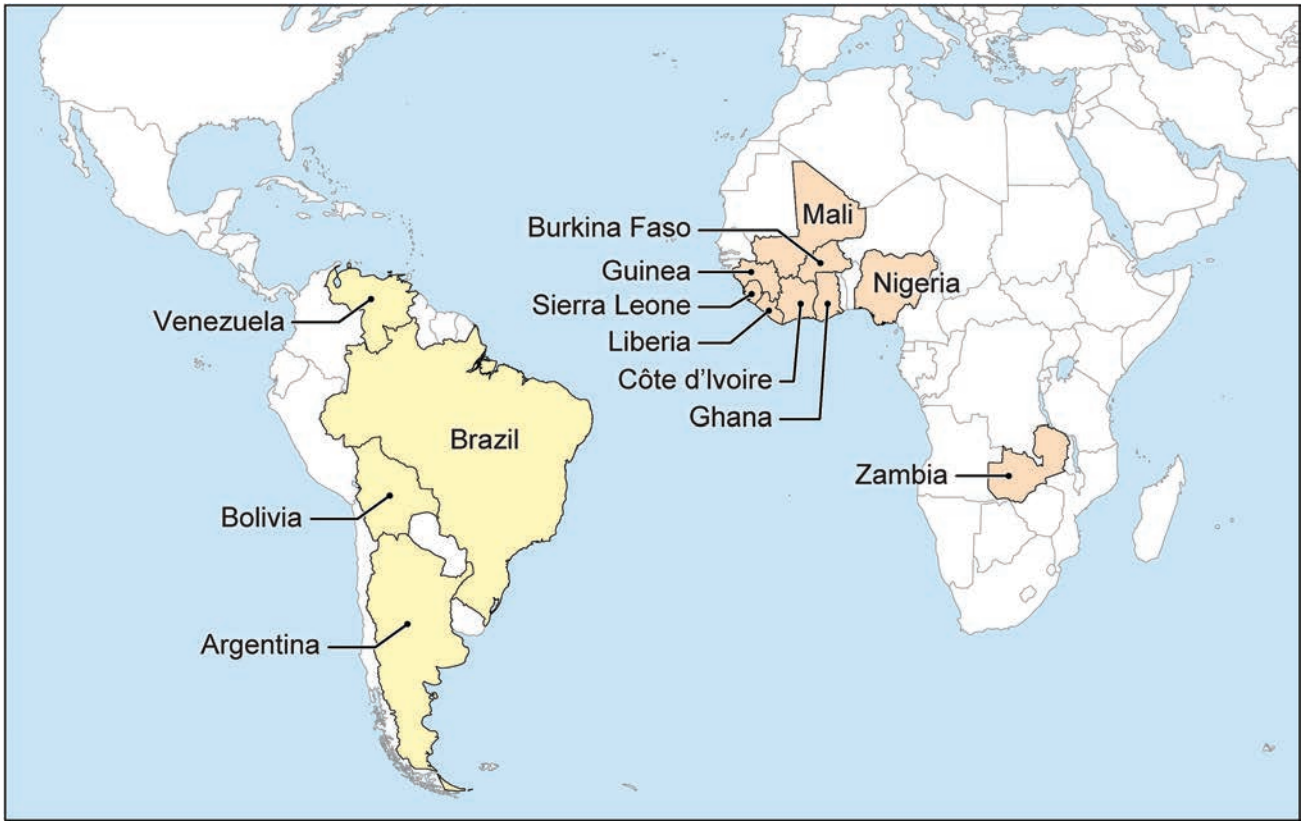

Figure 21-1. Global distribution of viral hemorrhagic fever-causing mammarenaviruses. Mammarenaviruses are phylogenetically and serologically differentiated into Old World (*orange*) and New World (*yellow*) groups. Old World mammarenaviruses: Lujo virus (Zambia) and Lassa virus (other highlighted countries). New World mammarenaviruses: Guanarito virus (Venezuela), Sabiá virus (Brazil), Chapare and Machupo viruses (Bolivia), and Junín virus (Argentina).

infection remains unknown. The patient was transferred to Johannesburg, South Africa, for medical management. Three secondary infections and one tertiary infection were reported, from which only one person survived.[3]

New World Mammarenaviruses

Argentinian hemorrhagic fever (AHF) cases were first described in the humid Pampas of Argentina in 1955 (Figures 21-1 and 21-2).[4] However, AHF epidemics may have occurred as early as 1943. The etiologic agent of AHF—JUNV—was later isolated from humans.[5,6] Since the 1950s, JUNV is estimated to have caused about 30,000 AHF cases. Without treatment, the case fatality rate is approximately 20%. The AHF-endemic region has expanded progressively into north-central Argentina to the extent that currently 5 million people are considered to be at risk of infection.[7]

Bolivian hemorrhagic fever (BHF) was recognized in 1959 on the island of Orobayaya in the Beni Department in northeastern Bolivia (Figures 21-1 and 21-3). However, it was not until 1964, after initial outbreaks of this emerging hemorrhagic fever (1959–1962) caused 470 cases, that BHF was first described by Mackenzie and coworkers.[8] MACV, the etiological agent of BHF, is named after a river close to the outbreak area. MACV was isolated in 1963 from the spleen of a fatal human case in San Joaquín.[9] Between 1962 and 1964, another series of localized BHF outbreaks occurred, which involved more than 1,000 patients, of whom 180 died. After 20 years of no reported cases, mainly as a result of rodent control measures,[9] an outbreak of 19 cases occurred in 1994. Eight additional BHF cases were recognized in 1999, and 18 cases occurred in 2000. A larger outbreak, with 200 suspected cases, occurred in 2008.[10] The case fatality rate of BHF is approximately 5% to 30%.

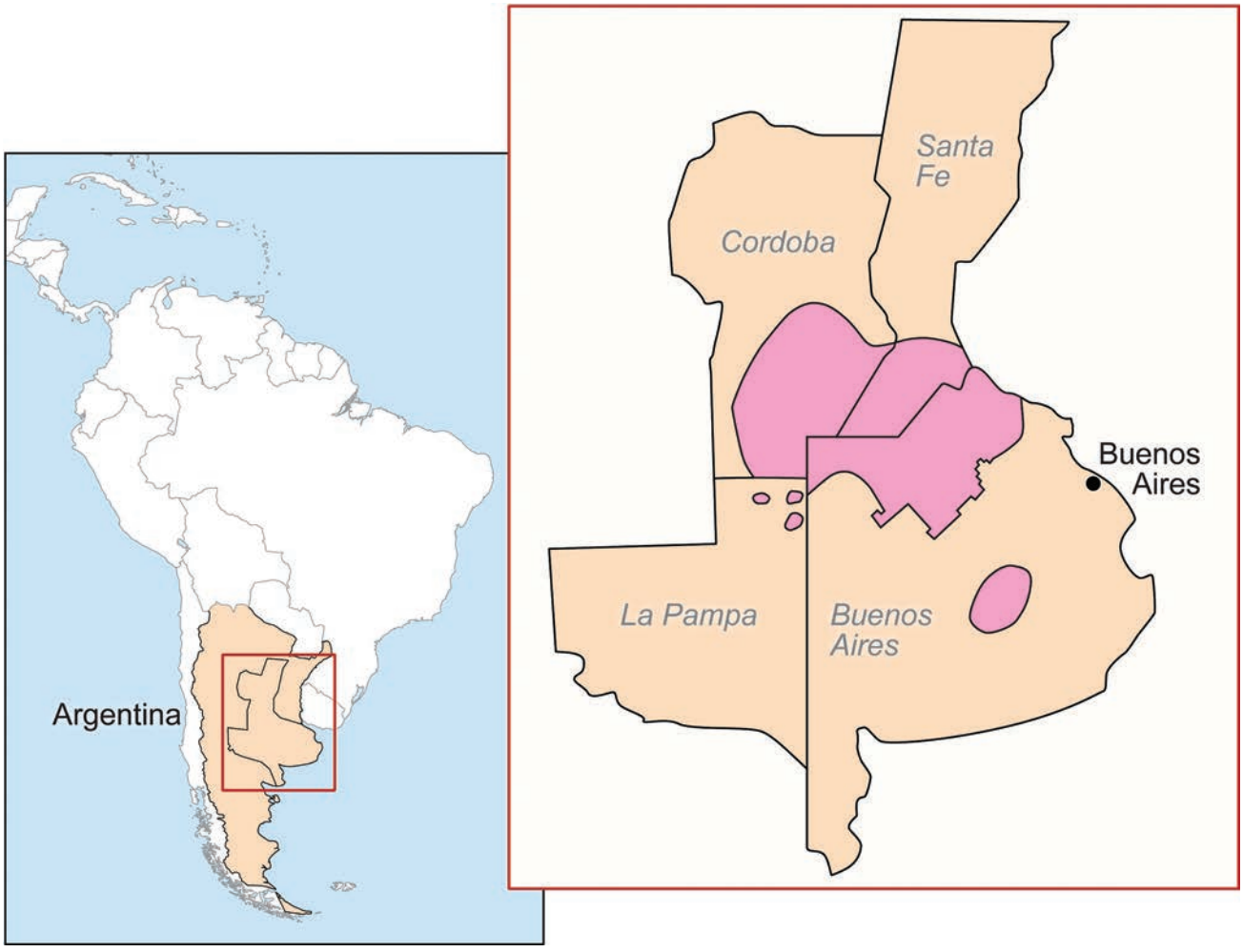

Figure 21-2. Geographic distribution of Junín virus in Argentina. Hyperendemic areas are shown in pink.

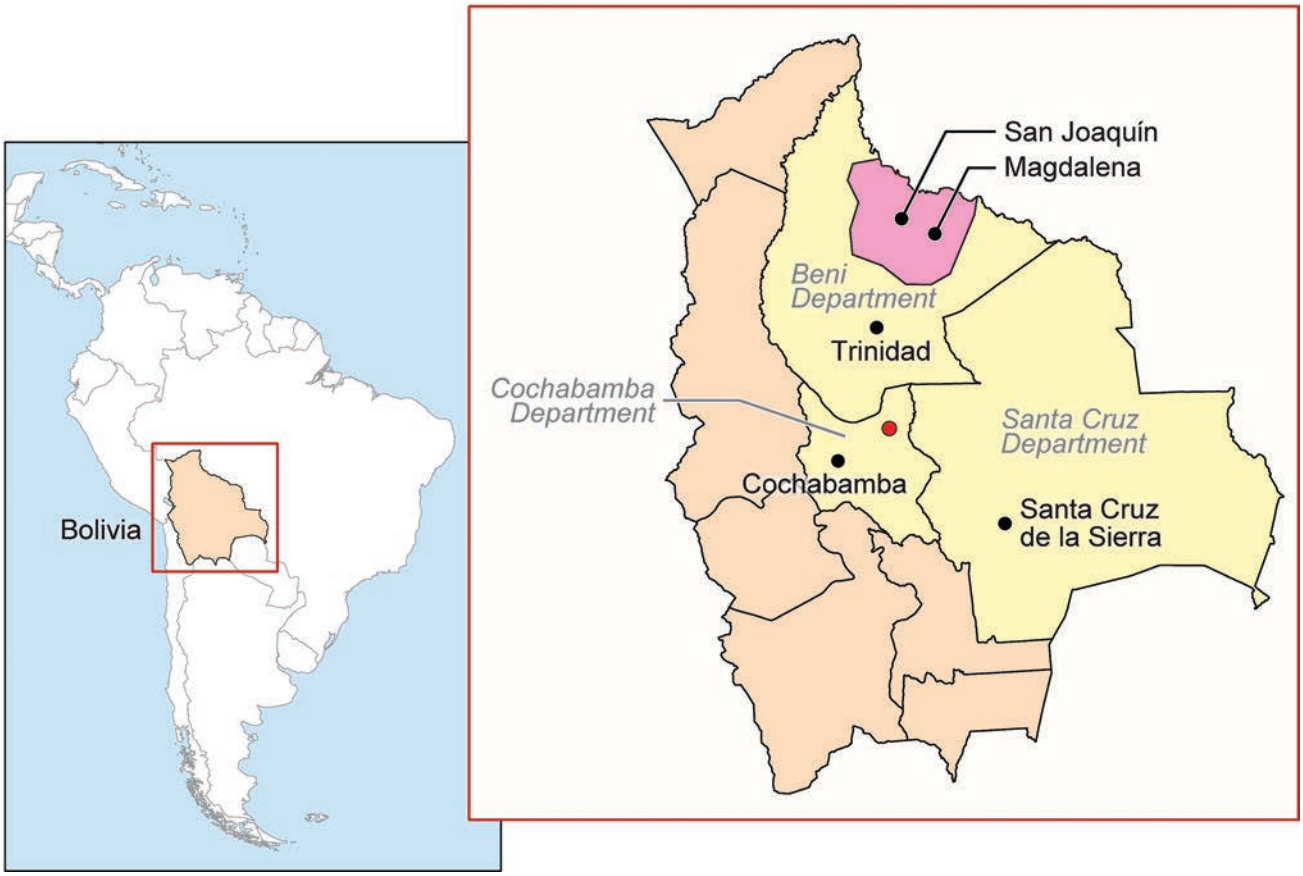

Figure 21-3. Geographic distribution of Machupo virus and Chapare virus in Bolivia (*yellow*). Hyperendemic areas are shown in pink. The only known location of Chapare virus infection is shown as a *red dot*.

GTOV emerged in 1989 as the cause of a yet officially unnamed disease that is often referred to as "Venezuelan hemorrhagic fever (VHF)." This severe hemorrhagic illness was recognized when settlers moved into cleared forest areas in the municipalities of Guanarito and Guanare in the state of Portuguesa in central Venezuela (Figures 21-1 and 21-4). The outbreak was initially misdiagnosed as severe dengue. Between 1990 and 1991, a total of 104 cases was reported with an approximately 25% case fatality rate.[11] The virus was isolated from the spleen of a 20-year-old male farm worker during autopsy.[12] After a seemingly spontaneous drop in human cases between 1989 and 1992, a new outbreak occurred in 2002 with 18 reported cases.[13] By 2006, approximately 600 cases of "VHF" have been reported.[14,15]

SABV, the cause of "Brazilian hemorrhagic fever," was isolated in 1990 from a single patient with a fatal infection in São Paulo, Brazil (Figure 21-1).[16] Subsequently, two laboratory infections were reported, one of which was successfully treated with ribavirin.[17]

In 2003 to 2004, CHAPV was recovered from a single fatal case of viral hemorrhagic fever in the Chapare River region in rural Bolivia (Figures 21-1 and 21-3). Additional cases were reported from this outbreak; however, details and laboratory confirmation are lacking.[18]

RESERVOIRS OF HEMORRHAGIC FEVER MAMMARENAVIRUSES

Rodents of the superfamily Muroidea are the natural hosts of most mammarenaviruses. Old World mammarenaviruses are found in rodents of the family Muridae, subfamily Murinae. New World mammarenaviruses are found in rodents of the family Cricetidae, subfamily Sigmodontinae. Bats may transmit Tacaribe virus, and reservoirs for Chapare, Lujo, and Sabiá viruses have not yet been identified.

The range of the corresponding rodent/bat host(s) determines the geographical distribution of each mammarenavirus. Field studies strongly support the concept of a single major reservoir host for each

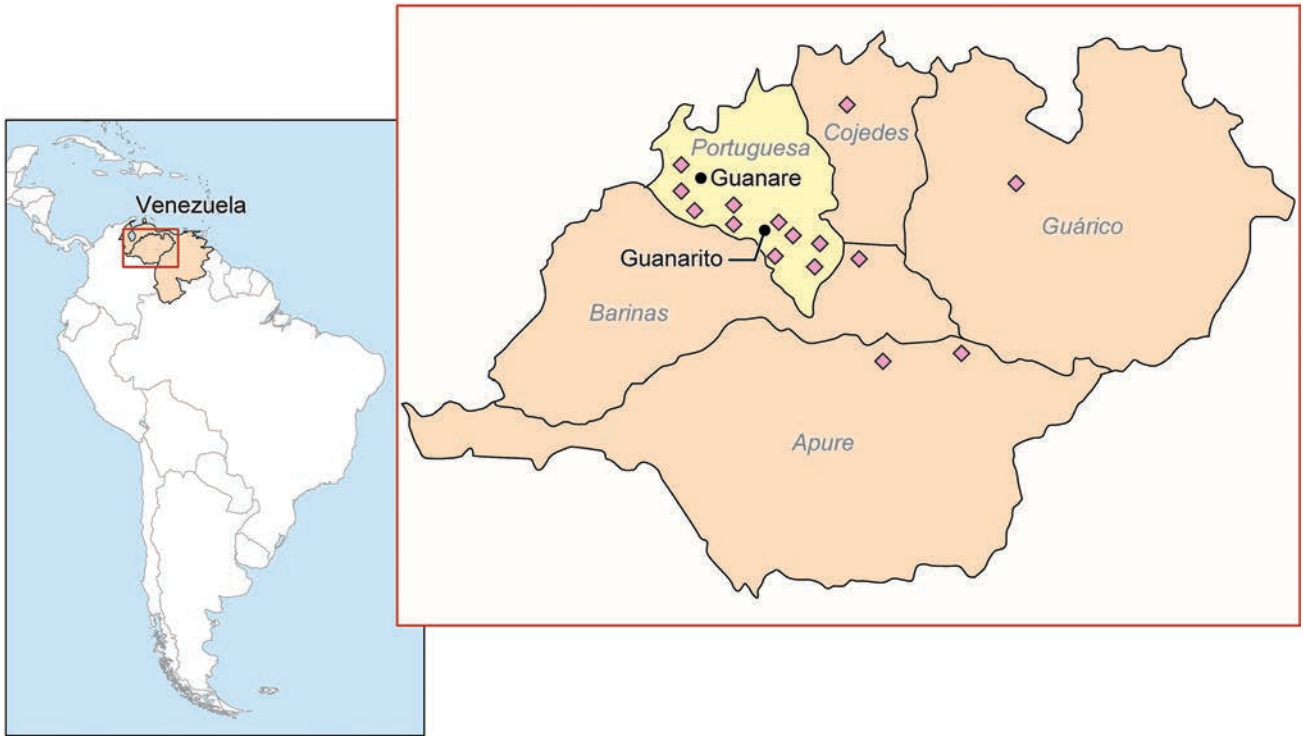

Figure 21-4. Geographic distribution of Guanarito virus in Venezuela. Clusters of infections areas are shown in *pink*.

virus.[19] Principal hosts for LASV, MACV, JUNV, and GTOV are the natal mastomys (*Mastomys natalensis*), the big laucha (*Calomys callosus*), the drylands laucha (*Calomys musculinus*), and the short-tailed zygodont (*Zygodontomys brevicauda*), respectively.[2,20–23]

Current evidence suggests a long-term "diffuse co-evolution" between mammarenaviruses and their rodent hosts. According to this model, a parallel phylogeny between the viruses and their corresponding rodent host(s) allows for host switches between closely related rodents.[19,24] Mammarenaviruses establish chronic infections in their respective reservoirs accompanied by chronic viremia or viruria without clinical signs of disease.[25–28]

Humans become infected with mammarenaviruses through contact with infected rodents or inhalation of aerosolized virus from contaminated rodent blood, excreta or secreta, or body parts caught in mechanical harvesters.[13] In Western Africa, peridomestic rodents are also part of the diet of inhabitants of LASV-endemic areas, and therefore contaminated meat may be another route of virus transmission.[29] New World mammarenavirus infections peak during harvest season when rodent populations are active. Infected cases are predominantly male agricultural workers who come in contact with infected rodents. Person-to-person transmission of LASV or MACV is not frequent, but it is possible by direct contact with body fluids or excreta of infected patients. Such transmission is probably not the principal mode of disease dissemination. Only small quantities of MACV can be isolated from human blood or from throat or oral swabs of infected patients.[30]

CLINICAL PRESENTATION

Old World Mammarenaviral Hemorrhagic Fevers

The signs and symptoms of Lassa fever vary depending on the disease's severity.[31,32] The disease is mild or asymptomatic in about 80% of infected people, but 20% develop acute Lassa fever. The incubation period can range from 1 to 24 days with an average of 7 to 18 days. Disease onset is insidious with low-grade fever, weakness, and general malaise. Within 2 to 4 days, many patients experience symptoms including myalgia; arthralgia; lower back, abdominal, and/or retrosternal pain; headache; dizziness; or sore throat. Hypotension, productive cough, vomiting, and diarrhea are also common. Pharyngitis or conjunctivitis can occur as the disease progresses, and mucosal bleeding (gums, nose, and other sites), pleural or

pericardial effusions, or facial or neck edema occur in more severe cases.[33] In the second week after onset, acute respiratory distress syndrome, moderate-to-severe diffuse encephalopathy, or shock develops in severe cases. Confusion, followed rapidly by tremors, convulsions, abnormal posturing, or coma, sometimes occur just before death.[34] Another neurological manifestation is unilateral or bilateral sensorineural deafness, which occurs in about 30% of convalescent patients.[33,35]

Lassa fever presents with symptoms and signs indistinguishable from those of other febrile illnesses, such as malaria or other viral hemorrhagic fevers. Therefore, Lassa fever is difficult to diagnose clinically, but it should be suspected in patients with fever (≥38°C) not responding adequately to antimalarial and antibiotic drugs. Fever, pharyngitis, retrosternal pain, and proteinuria are the most useful clinical predictors for a Lassa fever diagnosis. Fever, sore throat, and vomiting are the best predictors for negative outcome.[33] Disease outcome is also related to the degree of viremia and not to antibody response. The probability of fatal disease increases with high viremia, and survival rate is lowest in patients with both high viremia and high concentrations of aspartate aminotransferase. In patients recovering from Lassa fever, virus is cleared from blood circulation about 3 weeks after onset of illness.[36] Survivors of LASV infection often recover without sequelae. However, severe sensorineural hearing deficits, which may develop during disease, may persistent permanently in approximately 13% to 30% of survivors.[37–39]

Patients infected with LUJV initially present with symptoms and signs of nonspecific febrile illness such as severe headache, malaise, vomiting, fever, retrosternal pain, or myalgia. Disease manifestations increase in severity over 7 days with the development of diarrhea or pharyngitis. In some patients, morbilliform rash or facial edema is evident. Terminal features are acute respiratory distress syndrome, cerebral edema, neurologic signs, deteriorating renal function, or circulatory collapse. No overt hemorrhage is observed besides gingival bleeding, petechial rash, or oozing from injection sites in some of the patients.[3] However, the clinical description of disease caused by LUJV infection is currently based on the observation of only five patients.

New World Mammarenaviral Hemorrhagic Fevers

New World mammarenaviral hemorrhagic fevers caused by CHAPV, GTOV, JUNV, MACV, or SABV are clinically similar.[4,15,18,40–43] Disease begins insidiously after an incubation period of 1 to 2 weeks. Initial symptoms/signs often include fever and malaise, headache, myalgia, epigastric pain, or anorexia. After 3 to 4 days, signs become increasingly severe with multisystem involvement: prostration; abdominal pain; nausea and vomiting; constipation; or mild diarrhea. In some cases, dizziness, photophobia, retroorbital pain, or disorientation may also appear, as well as the earliest signs of vascular damage, such as conjunctival injection, skin petechiae, mild (postural) hypotension, or flushing over the head and upper torso. About 30% of patients develop more severe hemorrhagic or prominent neurologic manifestations (convulsions, tremor of the hands or tongue, coma) or secondary bacterial infections during the second week of illness. Hemorrhagic manifestations, such as bleeding from mucous membranes (gums, nose, vagina/uterus, gastrointestinal tract) and ecchymoses at needle puncture sites, are common in these patients. However, blood loss is minor overall. Capillary leakage is a hallmark of disease, and elevated hematocrit occurs during the peak of capillary leak syndrome.[13] Death usually occurs 7 to 12 days after disease onset from organ failure and shock.

Patients who survive begin to improve during the second week of disease onset. Convalescence often lasts several weeks with fatigue, hair loss, dizziness, or Beau's lines in digital nails. "VHF" convalescent symptoms also include sore throat or pharyngitis.[15,44,45]

TAXONOMY AND PHYLOGENETIC RELATIONSHIPS

The family *Arenaviridae* includes two genera, *Mammarenavirus* (mammalian arenaviruses) and *Reptarenavirus* (reptilian arenaviruses). The genus *Mammarenavirus* includes 31 species, and the majority of these species have only a single virus member each.[46,47] Based on antigenic properties (serological cross-reactivity), sequence phylogeny, and geographical distribution, mammarenaviruses have been divided into two distinct groups. The Old World mammarenaviruses (also named the Lassa–lymphocytic choriomeningitis serocomplex) include viruses indigenous to Africa, such as LASV and LUJV, as well as the ubiquitous lymphocytic choriomeningitis virus. The New World mammarenaviruses (also named the Tacaribe serocomplex) include viruses indigenous to the Americas,[48–51] such as CHAPV, GTOV, JUNV, MACV, and SABV.

The basis for mammarenaviruses phylogenetic analysis typically relies on the sequence of the nucleoprotein (N) gene. This analysis supports the previously defined antigenic grouping, further defines

virus relationships, and is largely consistent with analyses based on sequence data derived from other regions of mammarenaviruses genomes. According to N-based phylogenetic analysis, the member viruses of the 31 species represent four distinct phylogenetic groups: an Old World mammarenaviruses group and three New World mammarenavirus lineages (A, B, and C).[52–55] New World mammarenaviruses Group A includes Allpahuayo virus, Flexal virus, Paraná virus, Pichindé virus, and Pirital virus from South America, together with Bear Canyon virus, Tamiami virus, and Whitewater Arroyo virus from North America. Group B contains the human pathogenic viruses CHAPV, GTOV, JUNV, MACV, and SABV, and the nonpathogenic Amaparí virus, Cupixi virus, and Tacaribe virus. Group C includes Latino virus and Oliveros virus.

MOLECULAR CHARACTERISTICS

Arenaviruses produce enveloped and spherical to pleomorphic virions, ranging from 50 to 300 nm in diameter (Figure 21-5).[13,56–58] The particles' sandy appearance in electron microscopy sections earned these viruses their name (Latin arena = sand). The arenavirus genome consists of two single-stranded ribonucleic acid (RNA) molecules, designated L (large) and S (small). Each of these genomic segments encodes two different proteins in two nonoverlapping reading frames of opposite orientation (ambisense coding arrangement; Figure 21-5). The L segment encodes the viral RNA-dependent RNA polymerase (L) and a zinc-binding matrix protein (Z). The S segment encodes a nucleoprotein (NP) and an envelope glycoprotein precursor (GPC).[59–61] Extracted virion RNA is not infectious, and, therefore, arenaviruses are considered by some as negative-sense RNA viruses despite the presence of the ambisense coding strategy.

Arenavirus cell entry and fusion with the host membrane is mediated by the arenavirion spike complex (Figure 21-5). In the case of mammarenaviruses, the spike is composed of the two envelope glycoprotein subunits, GP1 and GP2, derived from posttranslational cleavage of GPC and a stable signal peptide, cleaved off during GPC synthesis.[61–65] Reptarenavirus spikes are fundamentally different from mammarenaviral spike proteins and are closely related structurally to the glycoproteins of filoviruses ($GP_{1,2}$). Therefore, a stable signal peptide is absent.[66] To enter cells, arenaviral GP1 binds to cell-surface receptors, and virions are internalized by endocytosis into intracellular endosomal compartments.[67–71] Following pH-dependent membrane fusion mediated by GP2 and uncoating, viral ribonucleoprotein (RNP) complexes are released into the cytoplasm (Figure 21-5).[58]

Interestingly, genes required for the proper functioning of α-dystroglycan (αDG), the receptor for Lassa virus (LASV), are preferential targets of LASV-driven selective pressure (or natural selection) in populations of Western Africa where LASV is endemic. A genome-wide screen for recent selective sweeps in humans has identified positive selection of two genes

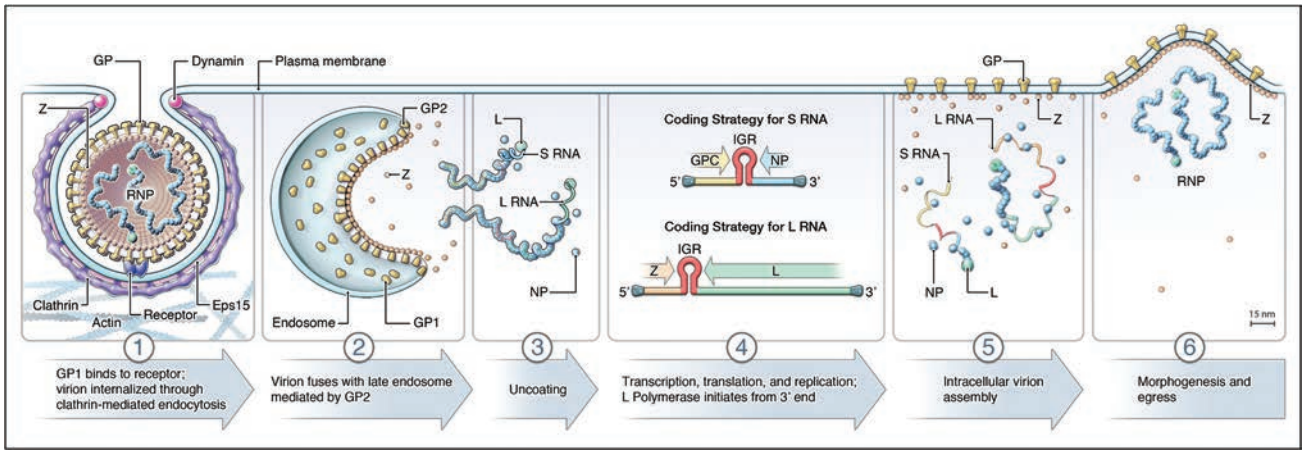

Figure 21-5. Replication cycle of an idealized mammarenavirus.
GPC: glycoprotein precursor
IGR: intergenic region
NP: nucleoprotein
RNA: ribonucleic acid

(LARGE and dystrophin) in a Nigerian population.[72–74] Dystrophin is a cytosolic adaptor protein that is necessary for αDG to function properly. The cellular like-acetylglucosaminyltransferase (LARGE) is required for the posttranslational modification of αDG and consequently for LASV GP binding to αDG.[75] In the identified population, more than 21% of individuals showed evidence of exposure to LASV infection, which may be responsible for the increase in the allelic variants of LARGE and dystrophin. Positive selection in LARGE was confined to introns and putative regulatory regions, suggesting that natural selection may have targeted variants giving rise to alternative splicing or differential gene expression of LARGE. These polymorphisms in LARGE and dystrophin may alter the ability of LASV GP to bind αDG and mediate entry, providing an advantage to the immune system, and thereby protecting these individuals from severe LASV infection.[72–74]

RNPs consist of NP, L, and viral genomic RNA. NP is the arenavirus major structural protein. It forms a bead-like polymer that associates with viral RNA. L, the arenaviral RNA-dependent RNA polymerase, mediates RNA replication and transcription (Figure 21-5).[76–79] L initiates transcription from the genome promoter at the genome 3′ end, and NP and L genomic complementary mRNA are synthesized and translated to proteins from the S and L segments, respectively.[80–82] L together with NP also generate a full-length copy of antigenome RNA from the L and S segments. Antigenome S and L RNA segments then serve as a template for the synthesis of GP and Z mRNAs, which are translated into the respective proteins. Newly synthesized full-length arenavirus antigenomic and genomic RNAs are encapsidated by NP to generate the RNP complexes for further mRNA transcription and for production of virus progeny (Figure 21-5).[83] The negative regulatory matrix protein Z inhibits arenaviral RNA synthesis in a dose-dependent manner.[82,84–88] Z contains a zinc-binding RING motif[89,90] that is essential for interaction with L and resultant inhibitory activity.[86]

Together with NP, Z mediates arenavirion assembly[91] and budding.[80,91,92] Virion budding occurs from the plasma membrane, where the virus RNP core associates with host-derived membrane that is highly enriched with the viral GP spike complex to form the virion envelope (Figure 21-5).[80,91–93]

In addition to the roles of NP and Z in viral replication, these proteins interfere with antiviral signaling. NP encoded by Old World and New World mammarenaviruses is involved in virus-induced inhibition of type I interferon.[94,95] Z protein encoded by New World mammarenaviruses also interferes with this pathway.[96]

PATHOLOGY AND PATHOGENESIS

The mechanism of pathogenesis of mammarenaviral hemorrhagic fevers is not well understood. In both Old World and New World mammarenavirus infections, the pathological findings do not provide the basis to explain the relatively high case fatality rate and severity of disease.[11,97–101] According to the current pathogenesis model, mammarenaviruses enter humans by inhalation and deposit in the lung terminal respiratory bronchioles. The viruses then gain entry to the lymphoid system and spread systematically in the absence of a detectable pneumonic focus.[102] Mesothelial surfaces are infected next, perhaps a source of some of the observed effusions of parenchymal cells of several organs, particularly lymphoid tissues. Macrophages are early and prominent targets of mammarenavirus infection.[17,103,104]

Old World Mammarenaviral Hemorrhagic Fevers

In the case of patients infected with LASV, failure to develop cellular and humoral immune responses, indicated by high levels of serum virus titers and virus replication in tissues, leads to the development of fatal Lassa fever.[105] Nonhuman primate models of Lassa fever indicate that dendritic cells are prominent targets of LASV in the initial stages of infection, whereas Kupffer cells, hepatocytes, adrenal cortical cells, and endothelial cells are more frequently infected with LASV in the terminal stages of infection.[106]

Macroscopic abnormalities in Lassa fever patients include pleural effusions, pulmonary edema, ascites, and hemorrhagic manifestations in the gastrointestinal mucosa.[1,31] Microscopic findings include multifocal hepatocellular necrosis and apoptosis or regeneration (mitosis), splenic necrosis in the marginal zone of the splenic periarteriolar lymphocytic sheath, adrenocortical necrosis, mild mononuclear interstitial myocarditis without myocardial fiber necrosis, alveolar edema with capillary congestion and mild interstitial pneumonitis, lymph nodal sinus histiocytosis, gastrointestinal mucosal petechiae, renal tubular injury, or interstitial nephritis.[1,31,107,108]

The most severe pathological hallmark of Lassa fever in humans is found in the liver.[1,31,107,109] In addition to hepatocellular necrosis, mononuclear phagocytosis of necrotic hepatocytes and focal hepatocellular cytoplasmic degeneration are typical. However, the degree of hepatic tissue damage is insufficient to cause hepatic

failure, and only minimal recruitment of inflammatory cells into this organ is detected. Furthermore, no correlation has been observed between the degree of hepatic necrosis and chemical indicators of liver damage, such as elevated concentrations of aspartate aminotransferase, alanine transaminase, and lactate dehydrogenase in serum.[107,108] Finally, the degree of liver damage can vary dramatically among patients that die from Lassa fever. Therefore, liver involvement is necessary—but not sufficient—in the chain of pathological events that lead to fatal Lassa fever.

Based on the degree of hepatic damage, three general phases have been proposed for the categorization of patients with fatal Lassa fever.[66] The first phase, active hepatocellular injury, is defined by the presence of focal cytoplasmic degeneration with less than 20% of hepatocytes undergoing necrosis. This phase may represent the late stage of viremic spread and early cellular injury. This phase is most likely caused by direct viral action rather than mediated by a cellular immune response, since lymphocytic infiltration is not detected. The second phase, the peak of Lassa hepatitis, is characterized by 20% to 50% necrosis of hepatocytes, widespread focal cytoplasmic degeneration, and limited phagocytic infiltration. Progressive hepatocellular damage occurs during this phase, but early liver recovery is evident through the phagocytic removal of necrotic hepatocytes and regeneration of new cells. The third phase, hepatic recovery, is defined by less than 10% of hepatocellular necrosis, absence of focal cytoplasmic degeneration, and clear evidence of mitoses, which indicates liver regeneration.[108]

In addition to hepatic necrosis, splenic and adrenocortical cellular necrosis is observed in patients with Lassa fever. The predominant distribution of splenic necrosis is in the marginal zone of the periarteriolar lymphocytic sheath. Close examination of thin tissue sections reveals the presence of fibrin in addition to the debris of necrotic cells. The splenic venous subendothelium appears to be infiltrated by lymphocytes and other mononuclear cells. Additionally, multifocal adrenocortical cellular necrosis is most prominent in the zona fasciculata and is often associated with focal inflammatory reaction. However, in all examined cases, adrenal necrosis was mild, and greater than or equal to 90% of the cells of adrenal cortex appeared viable.[107] Microscopic examination of adrenal glands shows prominent spherical, hyaline, and acidophilic cytoplasmic inclusions in cells near the junction of zona reticularis and medulla. In most cases, these cells appear to be adrenocortical cells of the zona reticularis; however, some cells are of adrenal medulla origin.

LASV-induced impairment of vascular function is most likely central for the pathology observed in infected patients. LASV, which is a nonlytic virus, does not cause cytopathic effects or cellular damage in infected monocytes, macrophages, and endothelial cells.[110] Nevertheless, infection of these cells is crucial for the pathology caused by the virus. In both experimentally infected animals and Lassa fever patients, the disruption of vascular endothelium function is closely followed by shock and death.[107,111] Edema and pleural and pericardial effusions that are associated with fatal cases most likely result from increased vascular permeability. Only minimal vascular lesions are detected in fatal human Lassa fever cases and infected nonhuman primates, which can be explained by the nonlytic nature of LASV infection of the endothelium. The mechanism of LASV-induced increase in vascular permeability is not yet understood. Virus infection of the endothelium is commonly thought to cause changes in cellular function leading to increased fluid flow and subsequently to edema.

New World Mammarenaviral Hemorrhagic Fevers

The most common macroscopic abnormality in severe cases of New World mammarenaviral hemorrhagic fevers is widespread hemorrhage, particularly in the skin and mucous membranes (gastrointestinal tract), intracranium (Virchow-Robin space), kidneys, pericardium, spleen, adrenal glands, and lungs. Microscopic lesions include acidophilic bodies and focal necroses in the liver (in the case of BHF, hepatic petechiae are common, and the number and size of the Kupffer cell are also increased), acute tubular and papillary necrosis in the kidneys, reticular hyperplasia of the spleen and lymph nodes, or secondary bacterial lung infections in the case of AHF (acute bronchitis and bronchopneumonia, myocardial and lung abscesses) or interstitial pneumonia in the case of BHF[97,98] or "VHF."[11,112] In AHF, the sites of cellular necrosis (hepatocytes, renal tubular epithelium, macrophages, dendritic reticular cells of the spleen and lymph nodes) correspond to sites of viral antigen accumulation, and both JUNV and MACV could be isolated from the blood, spleen, and lymph nodes of patients.[20,30,113,114]

Patterns of clinical AHF illness are JUNV strain-specific and can be hemorrhagic (Espindola strain), neurologic (Ledesma strain), mixed (P-3551 strain), and common (Romero strain).[115–117] In animal models of AHF, each isolate induces a disease that faithfully replicates the clinical variant of the disease in the human from whom the viral strain was obtained. Animals infected with JUNV Espindola strain (hemorrhagic) demonstrate a pronounced bleeding tendency with

disseminated cutaneous and mucous membrane hemorrhage. In contrast, animals infected with JUNV Ledesma strain (neurologic) show little or no hemorrhagic manifestations, but develop overt and generally progressive signs of neurologic dysfunction: limb paresis, ataxia, tremulousness, or hyperactive startle reflexes. In guinea pigs, the Espindola strain replicates predominantly in the spleen, lymph nodes, and bone marrow, the major sites of necrosis, whereas lower virus loads are present in blood and brain. The Ledesma strain, however, is found predominantly in the brain, where moderate polioencephalitis is observed, and only low amounts of virus are recovered from the spleen and lymph nodes.

COAGULOPATHIES

Although the mammarenaviruses discussed in this chapter cause viral hemorrhagic fevers in humans, blood loss does not typically account for the diseases' fatal outcome.[107] Furthermore, pathogenic mammarenaviruses differ in their capacity to cause hemorrhages or coagulopathies in infected individuals, which is particularly true in Lassa fever patients in whom bleeding is uncommon and limited primarily to mucosal surfaces.[118] In general, coagulation dysfunction is not considered to be associated with Lassa fever as neither disseminated intravascular coagulation nor a decrease in coagulation factors has been observed.[119] However, in the few severe cases characterized by bleeding, it is typically associated with moderate thrombocytopenia and platelet dysfunction.[119,120] The platelet malfunction appears to be mediated by a plasma inhibitor of platelet aggregation, which has yet to be characterized.[120]

Hemorrhages are more common in patients infected with New World mammarenaviruses than with Old World mammarenaviruses. However, similar to Lassa fever patients, relatively few vascular lesions are seen in patients with AHF.[121] Endothelial cells, which highly express the New World mammarenavirus receptor transferrin receptor 1, are permissive to infection in culture, but minimal cytopathic effects are observed.[70,122,123] Therefore, indirect effects may be responsible for the increased permeability seen in patients,[104] and profuse bleeding is presumably a consequence of vascular damage caused by both cytokines and virus replication. Thrombocytopenia, which is commonly found in patients with AHF and in animals experimentally infected with JUNV or MACV, and elevated concentrations of factor VIII-related antigen (von Willebrand factor, vWF), which is synthesized and released from endothelial cells, could contribute to the observed endothelial dysfunction.[124–128] However, vWF concentrations are low in JUNV-infected human umbilical vein endothelial cells, suggesting that vWF might originate from another source rather than from endothelial cells. Human umbilical vein endothelial cells infected with virulent JUNV strains increase production of the vasoactive mediators nitric oxide and prostaglandin PGI_2 compared to that observed with avirulent strains, providing a possible link between viral infection and the increased permeability observed in patients with AHF.[123]

In animal models of AHF and BHF, progressive thrombocytopenia is observed within 7 days following infection onset, with platelet counts reaching a nadir at or near the time of death. Coincident with the dropping platelet count, progressive necrosis of bone marrow occurs, suggesting that the decrease in the number of platelets may be related to impaired production.[101,117,127,128] Furthermore, intracytoplasmic viral particles have been demonstrated in megakaryocytes by electron microscopy.[129] The coagulative activity of blood in infected patients with AHF is also low.[130] Similar to Lassa fever patients, plasma from patients with AHF contains an inhibitor of platelet function.[35] Platelet inhibition appears to be reversible in vitro,[131,132] and its effects are not neutralized by immune plasma containing a high titer of (neutralizing) antibodies to JUNV. Thus, the available evidence suggests that abnormal platelet function in patients does not result from an intrinsic platelet defect, but rather from inhibition by an extrinsic factor in plasma.

Overall, specific coagulation abnormalities do not correlate with the severity of New World mammarenaviral infections, suggesting minimal involvement of coagulopathy in pathogenesis. Furthermore, limited evidence (four human cases)[98,133] suggests that disseminated intravascular coagulation is not an important pathogenic phenomenon in mammarenaviral disease. However, several modest abnormalities of clotting factors and activation of fibrinolysis are observed in AHF patients and animal models. These abnormalities can vary depending on which virus variant is involved.[124–126,128,134–136] The concentration of factor V is uniformly elevated (starting from day 8 of onset of AHF), and fibrinogen concentration is normal in mild cases and elevated in severe cases in the later stages of infection (after day 10). The activated partial thromboplastin time is prolonged during the acute phase of illness. A lower concentration ratio of factor VIII:C to vWF has been noted during the illness, but returns to normal during the convalescence period. In the guinea pig model, and to a lesser extent in humans, factor IX and XI concentrations are slightly

reduced.[124,126–128,130,134] Levels of prothrombin fragment 1+2 and thrombin-antithrombin III complexes are increased. However, antithrombin III activity levels in patients with AHF are within the normal range.[130] In another study in patients with AHF, antigenic and functional levels of antithrombin III were below normal in the early stages of disease.[137] In most cases of AHF, no significant changes in factor II, VII, prekallikrein, and kallikrein inhibitor, protein C, protein S, and C4b binding protein are observed.[124,130] Plasminogen activity is below normal in AHF patients in the earlier stages of the disease (days 6–11),[138] although normal or slightly elevated concentrations of α_2-antiplasmin can be detected.[124,130] Tissue plasminogen activator and D-dimer concentrations are high in the early stages of the disease, whereas the plasminogen activator inhibitor-I concentration is increased only in severe cases during the second week of illness.[130]

IMMUNE RESPONSE

Old World Mammarenaviral Hemorrhagic Fevers

Antibodies do not seem to play a significant role in LASV infection because production of LASV-specific antibodies is not correlated with Lassa fever survival. Such antibodies are detected in all patients, regardless of outcome.[36] Low neutralizing antibodies titers occur only months after an acute LASV infection is resolved, long after virus has been cleared.[139] Instead, resolution of LASV infection seems to depend primarily on cellular immunity, in particular the antiviral T-cell response.[140,141] Data from experimental nonhuman primate studies show a correlation between surviving animals and high concentrations of activated T lymphocytes and control of viral replication. In contrast, animals that died had delayed, low T-cell activation and uncontrolled viral replication.[142] In addition, seropositive individuals residing in Lassa fever-endemic areas have very strong memory $CD4^+$ T-cell responses, and the antigenic epitopes have been mapped to NP and an N-terminal conserved region within GP2.[141,143] However, results of a recent study using mice expressing humanized major histocompatibility complex class I and a single, exotic variant of LASV (Ba366) suggest that in the presence of persistent viremia, T cell responses may also contribute to deleterious innate inflammatory reactions and Lassa fever pathogenesis.[105] Whether these results can be generalized to other LASV variants remains to be determined.

In contrast to other viral hemorrhagic fevers, such as Ebola virus disease,[144] LASV infection does not result in a "cytokine storm" that interferes with the integrity of the vascular endothelium.[145] Virus-induced immunosuppression may be involved in the pathogenesis of severe Lassa fever. Antigen-presenting cells, such as dendritic cells and macrophages, and endothelial cells are early targets of LASV infection, with dendritic cells producing more virus than macrophages. However, these cell types are not activated to produce proinflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8).[110,146,147] These results are consistent with clinical data from Lassa fever patients showing correlation of fatal outcome and low concentrations or absence of proinflammatory cytokines such as IL-8 and interferon (IFN)-inducible protein 10.[145] Although increased vascular permeability does not seem to be caused by inflammatory mediators, LASV infection may affect endothelial cell integrity via another mechanism.

New World Mammarenaviral Hemorrhagic Fevers

Similar to Lassa fever, the acute phase of New World mammarenaviral hemorrhagic fevers is associated with significant depression of host immunity. The frequency of pyogenic secondary bacterial infections in humans and animal models[97,98,115] suggests that polymorphonuclear leukocyte function is compromised. Leukocyte dysfunction may be a result of bone marrow necrosis, maturation arrest, and direct interactions of JUNV with polymorphonuclear cells[148] that lead to leukopenia.[149,150] AHF is associated with a profound decrease in the recall of delayed hypersensitivity, diminished responsiveness of lymphocytes to nonspecific mitogens, decreased levels of circulating B- and T-cells, lymphoid necrosis, and inversion of $CD4^+/CD8^+$ lymphocyte ratios.[114,151–154] Abnormalities reported in animal models include necrosis of macrophages, T- and B-lymphocyte depletion, decreased primary and secondary antibody responses, and blunted Arthus reaction and anergy after established tuberculin sensitivity.[20,129,149,155–157] Defective macrophage function and high concentrations of IFN are highly plausible causes for these observed abnormalities. JUNV infects macrophages and monocytes extensively in vivo,[114] and circulating monocytes contribute to viral spread in the acute phase of AHF.[158] Virulent JUNV strains replicate in both spleen-derived dendritic cells and macrophages from guinea pigs, whereas attenuated strains, which are not immunosuppressive, replicate only in dendritic cells.[159]

Unlike LASV infection, in which generalized immune suppression is observed, AHF patients have elevated concentrations of proinflammatory as well as antiinflammatory cytokines that correlate with the disease's severity. Both patients and animal models

have high serum concentrations of IFN-α. The extraordinarily high serum concentrations of IFN-α in JUNV-infected patients are indicative of a negative disease outcome.[160,161] In patients who survive, high concentrations of IFN-α only occur during the first week after disease onset and fall to low concentrations during the second and following weeks. However, concentrations of interleukin-1β (IL-1β) remain normal.[102,160–162]

High serum concentrations of TNF-α, IL-6, IL-8, IL-10, and elastase-α1-antitrypsin complex are found in patients with AHF. Significant correlations are found between concentrations of both IL-8 and IL-10 with TNF-α concentrations, and between IL-8 and elastase-α1-antitrypsin complex. Thus, IL-8 is suggested to play an essential role in neutrophil activation in AHF patients.[163] Elevated TNF-α concentrations may be the trigger for some of the observed hemostatic and endothelial abnormalities observed in AHF patients. Results of several studies characterize the procoagulant activity[164–166] and changes in vascular permeability[167–169] of endothelial cells following exposure to TNF-α. JUNV–infected macrophages show no increase in cytokine production indicating that perhaps dendritic cells may increase cytokine concentrations.[170] At present, it is unclear if the increased cytokine concentrations reflect high viral replication and widespread infection or if they play a role in immune-mediated pathology similar to other viral hemorrhagic fevers like Ebola virus disease.

In contrast to Lassa fever, in which the antibody response seems to be ineffective in controlling LASV infection, patients who recover from AHF develop antibodies in the second week of disease and clear the virus.[171] In convalescent plasma from AHF survivors, robust titers of neutralizing antibodies, mainly immunoglobulin G (IgG), can be detected. After transfusion with immune plasma, viremia in patients is reduced.[172] The case fatality rate of AHF patients treated early during the course of infection with immune plasma from surviving cases is reduced to 1%.[173]

DIAGNOSIS

Detection of Virus-Specific Antibodies and Viral Antigens

Mammarenavirus antibodies can be detected by enzyme-linked immunosorbent assays (ELISAs), virus neutralization tests, and indirect immunofluorescence assays (IFAs). ELISAs using recombinant proteins, infected cells, or blood as antigen have been developed for detection of pathogenic mammarenavirus antibodies.[174–177] An immunoglobulin M (IgM) or IgG-specific ELISA is suitable for determining exposure to mammarenaviruses, but the relevance of IgM or IgG testing for acute infection depends on the virus and duration of illness.[176] An early immunosuppression resulting from Lassa fever seems to result in depressed production of IgM at the early phase of the infection and, as a result, some patients fail to elaborate IgM at the time of presentation. However, an increase in IgG titers is observed during convalescence. Thus, neither IgM nor IgG titers alone should be used as a screening tool for early detection of Lassa fever.

The virus neutralization assay is accepted as a standard serodiagnostic assay to quantify the antibody response to infection of a wide variety of viruses.[176] However, this test can be used for diagnosis of mammarenavirus infections only if a biosafety level 3 or 4 laboratory is available. Virus neutralization tests are highly specific, but neutralizing antibodies may appear too late in the course of mammarenaviral disease to be useful for prompt diagnosis. For example, patients with Lassa fever do not usually develop neutralizing antibodies until weeks after they became ill,[178] and patients with fatal Lassa fever may not develop, antibodies at all.[179]

IFA tests also detect antibodies in serum that bind to a fixed monolayer of virus-infected cells.[33,180] However, the interpretation of IFA is complicated by positive staining results in both the acute- and convalescent-phase of infection, as well as the subjective nature of the assay.[174] ELISAs are thought to be more sensitive and specific than IFA.[174,175,181,182] Cross-reactions can occur between different arenaviruses in these tests.

Compared to antibody detection, antigen-capture ELISA using polyclonal or monoclonal antibodies for detection of viral antigens is valuable for rapid diagnosis of acute phase viral hemorrhagic fevers, such as Lassa fever, AHF, BHF, "VHF," and "Brazilian hemorrhagic fever."[174,183] The sensitivity of sandwich antigen-capture ELISA is comparable to that of reverse transcription polymerase chain reaction (RT-PCR) for detection of Lassa fever.[184] In a comparison of the diagnostic markers in a large cohort of potential Lassa fever patients, LASV antigens detected in blood using antigen-capture ELISAs or lateral flow immunoassays are more indicative of an acute LASV infection than positive antibody titers.[177]

Detection of Viral Nucleic Acids

RT-PCR, real-time PCR, and real-time RT-PCR tests are valuable tools for rapid and early diagnosis of mammarenavirus infections.[177,179,185–192] However, the use of these assays in a clinical or environmental setting for the early detection of human cases has been limited by the expense of equipment and by expertise. RT-PCR has been used routinely for

confirmation of Lassa fever in Africa during collaborative missions following antigen detection by ELISA and lateral flow immunoassay.[177,193] Some PCR tests detect a wide range of mammarenaviruses by targeting the highly conserved termini of the S RNA segment, but an RT-PCR assay detecting Old World mammarenaviruses targeting the L gene has been also developed.[194] Other PCR tests are more virus-specific. Serum, plasma, cerebrospinal fluid, throat washings, and urine can be used for sample preparation. Real-time PCR may be advantageous because the risk of contamination is greatly reduced by using closed tubes and because the test quantifies viral RNA in serum. However, specimens containing a high concentration of viral RNA may produce false-negative results resulting from inhibition of the enzymatic reaction.[178] Given the high degree of genetic variability of mammarenaviruses, selection of primers that can detect all strains of the viruses can be difficult,[176] and PCR techniques may fail to amplify sequences of mammarenavirus strains even with limited sequence deviations.

Virus Isolation

Virus isolation is the gold standard for diagnosis of mammarenavirus infections.[174,177,193] Mammarenaviruses can easily be recovered in cell cultures, particularly from Vero cells. Initial passaging of a virus isolate in laboratory rodents, such as suckling laboratory mice, guinea pigs, or newborn hamsters, may be even more sensitive. The presence of virus can then be confirmed by PCR or by detection of virus antigen in cells using immunohistochemical or IFA assays. However, considering the time required for virus isolation (days to weeks) and the need for special facilities (biosafety level 3 or 4 laboratories), which are unavailable in many mammarenavirus-endemic areas, this method is less suitable for rapid diagnosis of mammarenaviral disease than PCR or antigen-capture ELISA.[174,177,195] Recent discoveries of novel mammarenaviruses relied on the use of IFA, PCR, and pyrosequencing technology.[3,18,194,196,197] Next-generation sequencing technology may be used in the near future for diagnostic purposes.

TREATMENT AND VACCINES

Few prophylactic and therapeutic treatments are approved for use against mammarenaviral hemorrhagic fevers. Treatment, therefore, consists primarily of supportive care and passive antibody therapy.

Passive Antibody Therapy

Transfusion of immune convalescent plasma with defined doses of JUNV-neutralizing antibodies is the present therapeutic intervention and treatment method against AHF. Immune serum treatment providing an adequate dose of neutralizing antibodies is effective in attenuating disease and reducing lethality to less than 1% if administered within the first 8 days of disease.[173,198–200] However, about 10% of treated patients develop a transient cerebellar-cranial nerve syndrome 3 to 6 weeks later.[173,198–200]

Studies with animal models suggest that passive antibody therapy may be useful for the treatment of BHF,[201] but such therapy has not been thoroughly evaluated in a clinical setting. An in vitro study with Vero E6 cells shows that convalescent sera from 6 of 7 putative "VHF" cases neutralized the infectivity of GTOV, and the neutralizing titers in the positive sera range from 160 to 640.[14] However, even if a similar plasma therapy could be developed for BHF and "VHF," maintaining adequate plasma stocks would be a challenge because of the limited number of cases and the absence of a program for convalescent serum collection. The additional risk of transfusion-borne diseases emphasizes that alternative treatments ought to be developed.[202]

In contrast, treatment of Lassa fever patients with convalescent serum of survivors did not confer protection when treated within 24 hours after hospital admission.[203] Treatment of nonhuman primates and guinea pigs with plasma from convalescent animals containing high titers of neutralizing antibodies protects the animals from developing disease. However, protection is observed only if administration of plasma is performed directly after infection with LASV.[204,205]

Vaccines

Despite the bioterrorism and public health risks associated with pathogenic mammarenavirus infection, FDA-licensed vaccines are currently not available. Vaccines for the prevention of human mammarenavirus diseases are limited to a single, safe, efficacious, and live attenuated vaccine designated Candid 1 (Candidate no. 1), for the prevention of JUNV infection.[200,206–208] Candid 1, which is classified as an investigational new drug in the United States, was derived from the wild type JUNV strain XJ13 through serial passage both in vivo and in vitro.[209] A recent study suggests that the major determinant of attenuation in mice is located in the transmembrane domain of the G2 glycoprotein (F427I mutation).[210] Candid 1 has been evaluated in

large-scale controlled trials among at-risk populations of agricultural workers in Argentina, where it showed a protective efficacy greater or equal to 84%. Vaccination of more than 150,000 high-risk individuals in the endemic areas has led to a consistent reduction in AHF cases with an excellent safety profile.[208,211,212] The vaccine also cross-protects experimental animals against MACV infection,[213] which suggests that Candid 1 could be used during a BHF outbreak as an emergency containment measure. A summary of the historical development and biological properties of the vaccine can be found in a recent review.[214]

Another approach for vaccine development against AHF involves using a nonpathogenic mammarenavirus relative, Tacaribe virus, as a live vaccine in guinea pigs or the marmoset model of AHF.[215–218] Animals develop measurable immune responses as early as 3 weeks following exposure to Tacaribe virus, and no clinical signs of AHF or histopathological changes are observed following exposure to a lethal dose of JUNV.

Several promising studies have focused on the development and preclinical testing of LASV vaccines. Nevertheless, no vaccine candidate has advanced toward human clinical trials. Early strategies involved the usage of an apathogenic mammalian Old World mammarenavirus, Mopeia virus, as a live vaccine. Rhesus monkeys that were inoculated with Mopeia virus and subsequently exposed to LASV developed no sign of LASV disease.[219,220] However, since little is known about human infections with Mopeia virus, and some of the infected primates developed pathological alterations of the livers and kidneys,[221] the safety of Mopeia virus should be proved before any efficacy studies are performed in humans.

Another live attenuated vaccine candidate against LASV infection is the chimeric ML29 virus. This recombinant virus carries the LASV S segment and the Mopeia virus L segment and is efficacious in nonhuman primates. Immunity is conferred via cellular responses, and no transient elevation of liver enzymes in the plasma is noted.[222,223] However, as in the case with candidate vaccines based on Mopeia virus only, caution must be exerted, as the safety of LASV-Mopeia chimeric vaccines in humans is unclear.

Recombinant viruses expressing mammarenaviral antigens have also been tested as potential vaccines. Different viral platforms, such as vaccinia virus, vesicular stomatitis Indiana virus, attenuated yellow fever strain 17D virus, and Venezuelan equine encephalitis virus replicon particles expressing mammarenaviral NP, GP, GP1, or GP2, have been evaluated in various animal models.[140,224–230] The most promising results were obtained using the whole GP of LASV.

Other approaches based on inactivated mammarenaviruses[231,232] or mammarenavirus-like particles[233] have not been successful or have yet to be fully evaluated, respectively.

Antiviral Agents

Current antimammarenaviral therapy is limited to an off-label use of the nonimmunosuppressive guanosine analogue, ribavirin (1-β-D-ribofuranosyl-1-*H*-1,2,4-triazole-3-carboxamide), an IMP dehydrogenase inhibitor. Recent studies suggest that the antiviral activity of ribavirin on mammarenaviruses is not mediated by depletion of the intracellular GTP pool, but may be exerted—at least partially—by lethal mutagenesis.[234–236] Unfortunately, ribavirin has only partial efficacy against some mammarenavirus infections and is associated with significant toxicity in humans.[17,200,203,212,237–244] Ribavirin can lead to adverse side effects such as thrombocytosis, severe anemia, and birth defects.[241,245]

Promising antivirals have been identified by small-molecule high-throughput screens. These antivirals can be divided into six chemically distinct classes of small-molecule compounds that specifically inhibit GP-mediated membrane fusion with different selectivities against New World and/or Old World mammarenaviruses.[236,246–248] One highly active and specific small-molecule inhibitor, ST-294, inhibits MACV, JUNV, GTOV, and SABV at concentrations in the nanomolar range. This molecule also demonstrates favorable pharmacodynamic properties (metabolically stable, orally bioavailable) and in vivo anti-mammarenaviral activity in a newborn mouse model.[246] Mechanism-of-action studies suggest that this compound is a viral entry inhibitor targeting GP2.[246] Another compound, ST-193, a benzimidazole derivative, inhibits cell entry of MACV and GTOV in vitro.[236] Finally, two lead compounds, 16G8 and 17C8, are highly active against MACV and GTOV, as well as LASV. These compounds act at the level of GP-mediated membrane fusion (IC_{50} ≈200–350 nM).[247] Despite chemical differences, evidence suggests that these diverse inhibitors act through the pH-sensitive interface of the signal peptide and GP2 subunits in the GP spike complex. The inhibitors prevent virus entry by stabilizing the prefusion spike complex against pH-induced activation in the endosome.[236,246,248]

Other types of inhibitors that target viral RNA synthesis have also been reported. T-705 (favipiravir), a pyrazine derivative with broad antiviral activity against RNA viruses[249–251] and several nonpathogenic mammarenaviruses,[252,253] is also active in vitro against MACV, JUNV, and GTOV. T-705 most likely acts as

a purine nucleoside analog specifically targeting the viral RNA-dependent RNA polymerase.[254] Results of studies using the Pichindé virus hamster model of acute mammarenaviral disease or a guinea pig model with an adapted Pichindé virus demonstrate that T-705 could effectively protect against mammarenaviral disease after onset of clinical signs or in the late stage of illness.[252,255]

SUMMARY

Arenaviruses represent a large and taxonomically diverse group of animal viruses that are maintained by small rodents, bats, and snakes in nature. The majority of arenaviruses is not known to cause disease in humans. Seven mammarenaviruses, however, are the etiological agents of severe viral hemorrhagic fevers associated with high case fatality rates. LUJV, CHAPV, GTOV, and SABV, which are geographically restricted, have been associated with only a few to a few dozen cases. They are, therefore, relatively unimportant to clinicians or the warfighter compared to many other viruses that are usually coendemic. However, MACV, JUNV, and especially LASV have caused large outbreaks (LASV has caused hundreds of thousands of infections per year). Visitors to countries in which these viruses are endemic, or warfighters that are deployed to these countries, need to be aware of how to prevent and suspect a mammarenavirus infection. Distance from or safe handling of rodents and their bodily fluids or tissues and general rodent control around human settlements or camps should be the first priority to prevent mammarenavirus infections.

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