Pathogenesis of the Viral Hemorrhagic Fevers

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Abstract

Four families of enveloped RNA viruses, filoviruses, flaviviruses, arenaviruses, and bunyaviruses, cause hemorrhagic fevers. These viruses are maintained in specific natural cycles involving nonhuman primates, bats, rodents, domestic ruminants, humans, mosquitoes, and ticks. Vascular instability varies from mild to fatal shock, and hemorrhage ranges from none to life threatening. The pathogenic mechanisms are extremely diverse and include deficiency of hepatic synthesis of coagulation factors owing to hepatocellular necrosis, cytokine storm, increased permeability by vascular endothelial growth factor, complement activation, and disseminated intravascular coagulation in one or more hemorrhagic fevers. The severity of disease caused by these agents varies tremendously; there are extremely high fatality rates in Ebola and Marburg hemorrhagic fevers, and asymptomatic infection predominates in yellow fever and dengue viral infections. Although ineffective immunity and high viral loads are characteristic of several viral hemorrhagic fevers, severe plasma leakage occurs at the time of viral clearance and defervescence in dengue hemorrhagic fever.

Keywords

filoviruses, dengue, yellow fever, arenaviruses, vascular permeability, hemorrhage
INTRODUCTION

The concept of viral hemorrhagic fever (VHF) was originated in the 1930s by Soviet investigators, who were studying hantaviral hemorrhagic fever (HF) with renal syndrome. These investigators later extended the designation to include Crimean–Congo HF and Omsk HF. The concept of VHF includes diseases caused by 23 enveloped RNA viruses from 4 taxonomic families: Arenaviridae, Bunyaviridae, Filoviridae, and Flaviviridae (Tables 1–4). The diseases vary from obscure geographically localized infections, such as Omsk HF and Kyasanur Forest disease, to highly publicized infections, such as Lassa fever in West Africa, that cause not only sporadic outbreaks such as Ebola HF (EHF) but also neglected endemic diseases that affect many people and constitute a true public health burden. Not all viruses in each family cause VHF (e.g., the arenavirus lymphocytic choriomeningitis virus does not cause such a disease), and not all patients infected with a VHF agent develop this syndrome (e.g., only 1% of persons infected with Rift Valley fever virus develop hemorrhagic manifestations and die).

The designation VHF is given to severe febrile illnesses with abnormal vascular regulation and vascular damage (1). The vascular dysregulation frequently manifests early in the course of illness as mild hypotension, flushing of the skin, postural hypotension, and vasodilation of the conjunctivae. As the disease progresses, vascular damage with capillary leakage may cause nondependent edema and serious effusions of body cavities such as the pleural and peritoneal spaces. Hemorrhages are more prominent in some diseases, such as Crimean–Congo HF, and occur infrequently in other infections, such as Lassa fever (even fatal cases). Hemorrhages usually occur, especially when the patient has thrombocytopenia or severe platelet dysfunction. These hemorrhages are seldom life threatening. In severe cases, vascular dysregulation and vascular damage with capillary leakage lead to shock, which is characteristic of the terminal phase of VHF.

The mechanisms of hemorrhage and plasma leakage in VHF include endothelial injury, activation of the mononuclear phagocytic system, secretion of pathologic concentrations of cytokines and other mediators, platelet aggregation and consumption, activation of the coagulation cascade, and insufficiency of coagulation factors arising from severe hepatic damage (2–4). The mechanisms vary among the diseases, the cell and organ tropism of the viruses, and the pathogenic and protective host responses. An understanding of the relative pathogenic roles of the viral agent and the host response to the infection is crucial; studies to elucidate these roles are under way. This review addresses VHFs caused by filoviruses, flaviviruses, and arenaviruses in detail.

FILOVIRUSES

Agents

Marburg virus was associated with a VHF that, in 1967, caused severe illness in people in Marburg, Germany, and Belgrade, Serbia (part of the former Yugoslavia). The patients had been exposed to tissues from nonhuman primates shipped from Uganda (5). Ebola virus subsequently appeared in both the Democratic Republic of the Congo (formerly Zaire) and Sudan, where highly lethal outbreaks occurred in 1976. The genetic diversity of the genus Ebola virus has led to the designation of five different species: Zaire ebolavirus, Sudan ebolavirus, Reston ebolavirus, Côte d’Ivoire ebolavirus, and tentatively Bundibugyo ebolavirus.

Filoviruses have a uniform diameter of 80 nm and form filaments 800–1,100 nm long that twist into various figures. The filovirus genome is a negative-sense, nonsegmented, single linear molecule of RNA that encodes seven genes: NP (nucleoprotein), VP35 (polymerase cofactor), VP40 (matrix protein), GP (glycoprotein), VP30 (transcription activator), VP24 (secondary matrix protein), and RNA-dependent RNA polymerase. There is a distinct difference between the Marburg virus GP gene, which encodes the single protein GP1,2, and the Ebola virus GP gene, which encodes soluble GP (sGP), Δ-peptide, and GP1,2.
Table 1 Filoviruses: agents, diseases, and epidemiology

<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Epidemiology</th>
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<tbody>
<tr>
<td>Marburg virus</td>
<td>Marburg hemorrhagic fever</td>
<td>Likely fruit bat reservoir, some outbreaks associated with caves or mines, nonhuman primates may be a common source of index patient infection, nosocomial spread in African hospitals lacking adequate procedures and supplies. Cases in the Democratic Republic of Congo, Kenya, Uganda, Angola, and South Africa.</td>
</tr>
<tr>
<td>Côte d’Ivoire ebolavirus</td>
<td>Ebola hemorrhagic fever</td>
<td>Human case after necropsy of dead chimpanzee. Case in Ivory Coast.</td>
</tr>
<tr>
<td>Bundibugyo ebolavirus</td>
<td>Ebola hemorrhagic fever</td>
<td>Cases in Uganda.</td>
</tr>
<tr>
<td>Reston ebolavirus</td>
<td>Asymptomatic infection</td>
<td>Infections in pigs in Philippines. Infected nonhuman primates imported into United States and Italy.</td>
</tr>
</tbody>
</table>

Clinical Manifestations

Marburg disease and EHF have occurred in outbreaks in Africa or following the entry of travelers from Africa into North America or Europe; the original outbreak was a nonhuman primate–associated epidemic in Europe. The desperate conditions in underdeveloped African hospitals have not allowed for extensive collection of clinical and laboratory pathophysiologic observations. There have been 17 outbreaks of EHF, with a 78% case-fatality rate for

Table 2 Flaviviruses: agents, diseases, and epidemiology

<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Epidemiology</th>
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<tbody>
<tr>
<td>Dengue viruses (types 1–4)</td>
<td>Dengue hemorrhagic fever, dengue shock syndrome</td>
<td>A–human cycle. Southeast Asia, Pacific rim tropics and subtropics, Caribbean islands, tropical and subtropical South America, Central America, Mexico, Florida, and Texas.</td>
</tr>
<tr>
<td>Omsk hemorrhagic fever virus</td>
<td>Omsk hemorrhagic fever</td>
<td>Undefined zoonotic cycle of ticks, muskrats, and voles. Potential transmission by mosquitoes and water. Western Siberia.</td>
</tr>
<tr>
<td>Kyasanur Forest disease virus</td>
<td>Kyasanur Forest disease</td>
<td>Tick-mammal-tick cycle. Tick transmission. Rural Karnataka state, India.</td>
</tr>
<tr>
<td>Alkhumra virus</td>
<td>Unnamed</td>
<td>Undetermined, possibly tick-livestock cycle.</td>
</tr>
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Table 3  Arenaviruses: agents, diseases, and epidemiology

<table>
<thead>
<tr>
<th>Agent</th>
<th>Disease</th>
<th>Epidemiology</th>
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<tbody>
<tr>
<td>Junin virus</td>
<td>Argentine hemorrhagic fever</td>
<td>Natural outbreaks occur during the major harvest season. Cases are reported only in Argentina. The primary host for Junin virus is the drylands vesper mouse.</td>
</tr>
<tr>
<td>Machupo virus</td>
<td>Bolivian hemorrhagic fever</td>
<td>Tends to be a seasonal disease. More cases occur in the dry season at the peak of agricultural activity. The reservoir is a species of the genus <em>Calomys</em>. Epidemics in towns may occur during epizootic conditions when rodent densities reach unusually high levels. Person-to-person transmission, probably via direct contact with infectious body fluids, may occur in familial or nosocomial settings.</td>
</tr>
<tr>
<td>Guanarito virus</td>
<td>Venezuelan hemorrhagic fever</td>
<td>This particular pathogen is specifically associated with <em>Zygodontomys brevicauda</em>, whereas a second arenavirus, Pirital virus, is associated with sympatric <em>Sigmodon alstoni</em>. Most epidemics occur from November to January, coinciding with increased agricultural activity.</td>
</tr>
<tr>
<td>Sabia virus</td>
<td>Brazilian hemorrhagic fever</td>
<td>Human infection in Brazil. The reservoir is unknown.</td>
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<tr>
<td>Lassa fever virus</td>
<td>Lassa hemorrhagic fever</td>
<td>Epidemics of Lassa fever occur in parts of West Africa, including Liberia, Guinea, Sierra Leone, Nigeria, and the Central African Republic. The virus causes lifelong infection in rodents of <em>Mastomys</em> species (multimammate rats) that are distributed over large parts of Africa.</td>
</tr>
<tr>
<td>Lujo virus</td>
<td>Unnamed disease</td>
<td>A chain of human cases originated in Zambia. The reservoir is unknown.</td>
</tr>
<tr>
<td>Chapare virus</td>
<td>Unnamed disease</td>
<td>Human cases in Bolivia. The reservoir is unknown.</td>
</tr>
</tbody>
</table>

*Zaire ebolavirus* and a 53% case-fatality rate for *Sudan ebolavirus* (6, 7). The single case of *Côte d’Ivoire ebolavirus* survived. The case-fatality rate of the sole *Bundibugyo ebolavirus* outbreak was 25%. Although Marburg disease is considered to be less severe than EHF, its case-fatality rate is 82%. Notably, the case-fatality rate of patients with Marburg disease receiving care in Europe and the United States was only 24% compared with 83% in the Democratic Republic of Congo and 90% in Angola. Human *Reston ebolavirus* infections have been subclinical.

In the outbreak of EHF in Kikwit, Democratic Republic of Congo, the early clinical manifestations were fever, headache, myalgia, diarrhea, vomiting, and abdominal pain (8, 9). In the terminal state, patients were obtunded and developed tachypnea, anuria, and shock, and their body temperature fell to a normal or subnormal level. The incubation period was 5 to 8 days, and death occurred a mean of 10.2 days after onset. Hemorrhagic manifestations were observed in only 41% of patients.

The variable manifestations of hemorrhage included petechial rash, which is often not visible in darkly pigmented skin; conjunctival bleeding; epistaxis; melena; and hematemesis (6, 10). General observations include an abrupt onset and a fatal course characterized by shock, fluid redistribution, disseminated intravascular coagulation, and the absence of an antibody response to the virus (10).

**Epidemiology**

Marburg disease outbreaks, which have occurred on average once every five years...
Table 4  Bunyaviruses: agents, diseases, and epidemiology

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<tr>
<th>Agent</th>
<th>Disease</th>
<th>Epidemiology</th>
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<tbody>
<tr>
<td>Crimean–Congo hemorrhagic fever virus</td>
<td>Crimean–Congo hemorrhagic fever</td>
<td>Natural tick-mammal-tick cycle and transovarian maintenance in <em>Hyalomma</em> ticks. Humans infected by tick bite, aerosols from or contact with slaughtered ruminants in the Middle East, southeastern Europe, and western China.</td>
</tr>
<tr>
<td>Rift Valley fever virus</td>
<td>Rift Valley fever</td>
<td>Transovarian maintenance in <em>Aedes</em> mosquitoes with episodic amplification by horizontal transmission involving many mosquito species, cattle and sheep. Humans infected by mosquito bite and contact or aerosol exposure to sheep, goats, and cattle in Africa and the Arabian Peninsula.</td>
</tr>
<tr>
<td>Hantaan, Puumala, Seoul, and Dobrava viruses</td>
<td>Hemorrhagic fever with renal syndrome</td>
<td>Horizontal cycles in Muriniae and Avicolineae rodents. Humans infected by aerosols of rodent urine predominantly in Eurasia.</td>
</tr>
<tr>
<td>Sin Nombre, Andes, Choclo, and other viruses</td>
<td>Hantavirus cardiopulmonary syndrome</td>
<td>Horizontal cycles in sigmodontine rodents. Humans infected by aerosols of rodent urine in North, Central, and South America.</td>
</tr>
<tr>
<td>Severe fever with thrombocytopenia syndrome virus</td>
<td>Severe fever with thrombocytopenia syndrome</td>
<td>Undefined natural cycle with likely transmission to humans probably by <em>Haemaphysalis longicornis</em> tick bite in China.</td>
</tr>
</tbody>
</table>

since 1975, have occurred in the Democratic Republic of Congo, Angola, Uganda, Kenya, and South Africa (6, 7). Some outbreaks have involved workers in gold and lead mines. EHF has occurred in the Democratic Republic of Congo, Sudan, Uganda, the Republic of Congo, Gabon, and the Ivory Coast. Infections have been associated with exposure to carcasses of nonhuman primates, the Ebola virus–associated die-off of which threatens some species. One outbreak of EHF in the Democratic Republic of Congo was associated with contact with and eating of bats.

Filoviruses have been detected in fruit bats and insectivorous bats in Africa (6). *Zaire ebolavirus* and antibodies to the virus have been found in three tree-roosting species of fruit bats. Marburg virus was recovered from a cave-inhabiting fruit bat, *Rousettus aegyptiacus*. Reston *ebolavirus*, which has caused infections in colonies of nonhuman primates in the Philippines, has been detected in pigs in that country. Reston *ebolavirus*–infected nonhuman primates have been imported into the United States.

However, the primary source of human infections with filoviruses has been underfunded African hospitals, which have inadequate supplies and personnel who may lack sufficient knowledge of infection control. Outbreaks have occurred when an infected patient is admitted to a hospital, where spread occurs through, for example, the reuse of contaminated needles. Transmission also seems to occur by contact with the skin of patients and cadavers.

Interaction Between Viral Proteins and Cells

Viral glycoprotein precursor (GPC) is cleaved in the trans-Golgi network into disulfide-linked GP1 and GP2. GP1 binds to the unknown host cell receptor, and GP2 mediates fusion of the viral and cell membranes. Filoviral entry mechanisms include (a) endocytosis that is receptor mediated or dependent on lipid rafts and (b) macropinocytosis (11). An alternative entry mechanism is mediated by clathrin.
A separate, related protein, sGP, serves an anti-inflammatory, endothelial barrier protector function. Virus-like particles composed of GP1,2 and VP40 activate endothelial cells to express intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin, and they increase endothelial permeability, which is further increased by tumor necrosis factor α (TNF-α) (12). These virus-like particles induce macrophages to upregulate the expression of messenger RNA of TNF-α, interleukin (IL)-6, IL-8, and IL-1β, as well as the secretion of TNF-α, IL-6, and IL-8 (13). Ebola surface GP also induces cytopathic effects, including rounding of the cells, increased membrane permeability, and detachment.

VP35 and VP24 inhibit type I interferon (IFN) activity. The 5′ triphosphate ends of genomic filoviral RNA represent a pathogen-associated membrane pattern that can be detected by RIG-I (retinoic acid–inducible gene 1) induction of a type I IFN response. VP35 inhibits induction of the IFN-β production by suppressing phosphorylation and dimerization of IFN regulatory factor 3 (IRF-3) and enhancing SUMOylation of IRF-7. VP35 blocks IFN-α production by inhibiting double-stranded RNA-dependent protein kinase. In addition, the viral VP24 inhibits type I and type II IFN signaling by inhibiting nuclear STAT1 (signal transducer and activator of transcription 1). There are a few differences between the antiviral function of these proteins and that of other viral proteins among filoviruses; however, all these viruses inhibit the potential production and the biological activity of IFN.

**Human Pathology and Pathogenesis**

Filoviruses cause extensive, severe pathologic lesions (14). Hemorrhagic lesions include petechiae and ecchymoses of skin, mucous membranes, and visceral organs, and large effusions occur in body cavities. Multifocal necrosis occurs most severely in liver, spleen, kidneys, testes, and ovaries (15). There is minimal inflammatory response to the necrosis. Hepatic lesions include hepatocellular necrosis and apoptosis, microvesicular steatosis, and Kupffer cell hyperplasia. Eosinophilic oval or filamentous cytoplasmic inclusions represent aggregates of Ebola virus nucleoproteins. Lungs contain hemorrhages and diffuse alveolar damage. Viral infection at the time of death involves macrophages and endothelial cells.

Three studies of plasma concentrations of cytokines, chemokines, growth factors, and inflammatory mediators differed in terms of the analytes and methods used, as well as many of their results (8, 16, 17). Overall, these studies favored excess production of IL-1 receptor antagonist (IL-1RA), IL-6, TNF-α, and IL-10 in fatal cases. The most extensive investigation in terms of the numbers of analytes and patients reported increased concentrations of proinflammatory IL-1β, IL-8, IL-15, IL-18, macrophage inflammatory protein (MIP)-1α, MIP-1β, monocyte chemotactic protein (MCP)-1, macrophage colony-stimulating factor, migration inhibitory factor (MIF), IFN-γ-induced protein 10 (IP-10), growth-regulated protein α (GRO-α), and eotaxin, as well as very low concentrations of T cell cytokines, IL-2, IL-3, IL-4, IL-5, IL-9, and IL-13 in fatal cases. Important differences in the reported data included whether or not plasma levels of IFN-γ, TNF-α, and IFN-α are elevated. The largest study found no increase in antiviral IFN-α2 (16). A study of a limited number of patients found decreased circulating CD4 and CD8 T lymphocytes, which was associated with a large increase in Fas in the T lymphocytes; this finding suggested massive loss by apoptosis mediated by Fas/FasL (Fas ligand) (16). Fatal human EHF cases do not mount an effective immune response and do not control viral replication. Survivors develop immunoglobulin G (IgG) antibodies mainly against viral nucleoprotein early in the course of illness; thereafter, the cytotoxic T cells are activated. In contrast, terminally ill patients never develop IgG antibodies, and only one-third of these patients mount a weak IgM antibody response (18). Killer Ig-like receptors (KIR2DS1

**IRF:** IFN regulatory factor
and KIR2DS3), human genes associated with a fatal outcome (19), are membrane proteins expressed on the surface of natural killer (NK) and T cells (19). They play a role in driving NK cell function, and an activating killer Ig-like receptor profile may play a role in a fatal outcome.

Limited clinical laboratory data reveal leukopenia with lymphopenia on presentation and subsequent leukocytosis and thrombocytopenia (10). Hepatic injury is detected by increased serum levels of aspartate and alanine aminotransferases. By the end of the first week of illness, renal impairment appears as oliguria, increased serum concentrations of urea and creatinine, and renal failure in fatal cases. Coagulopathy with prolonged prothrombin and partial thromboplastin times often meet the criteria of disseminated intravascular coagulation, which include elevated plasma D-dimer concentration.

**Animal Models**

Experimentally infected mice, guinea pigs, and nonhuman primates have been studied as models of EHF and Marburg HF. Rhesus and cynomolgus macaques provide the closest models of the human filovirus infections. The primary target cells of filoviruses are dendritic cells, monocytes, macrophages, and Kupffer cells. Although lymphocytes are not infected, bystander lymphocytic apoptosis occurs even at early stages of the infection. During the course of infection, there is marked depletion of NK cells and CD8 and CD4 T lymphocytes by apoptotic cell death (20).

The nonhuman primates are highly susceptible to filoviruses, and experimental infections have usually employed lethal doses. Cynomolgus monkeys infected with Marburg virus develop fever, anorexia, and rash on days 5–7 after inoculation and manifest leukocytosis characterized by neutrophilia and lymphopenia; decreased platelet counts; prolonged prothrombin and partial thromboplastin times; the presence of and a dramatic rise in circulating D-dimers, which indicate disseminated intravascular coagulation; and terminal decrease in activated protein C concentration (21). Lymph node and spleen enlargement is accompanied by a proinflammatory cytokine response (increased levels of IFN-α, IFN-β, IL-6, IL-8, IL-12p40, IL-12p70, IL-13, TNF-α, MIP-1α, MIP-1β, MCP-1, eotaxin, IL-1R, and IL-2R). At days 7 and 8, increased numbers of virus-infected cells appear in the lymph node paracortex, which is depleted by lymphocytic apoptosis. Lymphoid depletion and apoptosis are observed on days 6 and 7 in the spleen, as is the deposition of fibrin in the red pulp and marginal zone of the periarteriolar lymphocytic sheath. Decreased DC-SIGN (dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin) antigen in spleen and lymph nodes corresponds to decreased dendritic cell function. In the liver, Marburg virus first infects Kupffer cells and subsequently spreads to hepatocytes, which undergo apoptosis and necrosis.

Ebola virus infection of cynomolgus monkeys follows a similar course; early and sustained dendritic cell infection is associated with upregulated TRAIL (TNF-related apoptosis–inducing ligand) (22). Apoptosis occurs in bystander CD4 and CD8 lymphocytes and NK cells (23). Animals develop high viral titers in all organs, along with liver injury, renal failure, and disseminated intravascular coagulation. Infected mononuclear cells, tissue macrophages, endothelial cells, and neutrophils express increased quantities of tissue factor. Increased concentrations of D-dimers and tissue plasminogen activator, and decreased concentrations of activated protein C and platelets, are accompanied by fibrin thrombi in the splenic marginal zone, hepatic sinusoids, and renal medulla (24). Fibrin is closely associated with Ebola virus–infected cells. Decreased serum albumin concentration on days 5 and 6 reflects increased vascular permeability, but endothelial infection occurs late in the course of the disease and is focal; the endothelium remains relatively intact (25).

Rhesus monkeys infected with Ebola virus also develop fever, macular rash, petechiae, leukocytosis, lymphopenia, thrombocytopenia,
disseminated intravascular coagulation, and cytokine-associated systemic inflammatory response syndrome (10, 26). A telemetry study revealed that a fatal course is characterized by decreased mean arterial blood pressure, tachycardia, tachypnea corresponding to metabolic acidosis, acute renal failure, and hypothermia. Treatment with intravenous saline resulted in less severe renal impairment but did not have a survival benefit (27).

**Viral Entry, Targets, and Spread**

Although large outbreaks have been associated with nosocomial transmission, such as the reuse of inadequately sterilized needles, natural infections probably occur following filoviral entry via mucosal surfaces, such as the conjunctiva and oropharynx, or injured skin (28). Direct contact with human patients, cadavers, or naturally infected nonhuman primates may lead to exposure to viral particles from the skin or secretions. Exposure to and eating of freshly killed bats have been associated with Ebola virus infection. The primary targets are macrophages and dendritic cells. Hematogenous and possibly lymphatic vascular spread leads to the infection of mononuclear phagocytes in nearly every organ. Lymph nodes, spleen, and liver contain high titers of virus owing to extensive replication in these sites.

**Pathogenic Mechanisms**

Infection of human monocytes with Ebola or Marburg virus activates these cells and triggers the secretion of proinflammatory cytokines (IL-1β, TNF-α, and IL-6) and chemokines (IL-8 and GRO-α) (29). Moreover, GP1,2–containing virus–like particles (but none of the sGP products) induce high levels of human monocytic activation and secretion of TNF-α, IL-6, and GRO-α. The virus–like particles containing Ebola virus GP1,2 induce decreased endothelial barrier function, which is exacerbated by TNF-α. TNF-α released by filovirus-infected mononuclear phagocytes plays a role in the mediation of increased endothelial permeability (30). Mediators from filovirus-infected mononuclear cells cause reorganization of endothelial function, which results in interendothelial gaps that are associated with tyrosine phosphorylation of PECAM-1 (platelet endothelial cell adhesion molecule) but not of VE-cadherin–catenin complex proteins (31). Thus, the loss of endothelial barrier function in filoviral infections is caused by mediators’ effects on endothelium, rather than extensive filoviral infection of endothelial cells.

Ebola virus–infected cells secrete TRAIL, and increased sFas can then be detected. However, the pathway and mechanism of massive apoptosis of CD8 and CD4 T lymphocytes and NK cells have yet to be elucidated (32).

The extensive multifocal necroses in filoviral infections are probably caused, at least in part, by ischemia associated with fibrin thrombi caused by disseminated intravascular coagulation. Treatment of rhesus monkeys infected with a lethal dose of Ebola virus with recombinant human activated protein C led to the survival of 2 of 11 animals and prolonged life for all the infected animals (33). The survivors had lower concentrations of D-dimers, IL-6, IL-10, MCP-1, and TNF-α, as well as lower viral loads. Treatment of Ebola virus–infected monkeys with recombinant nematode anticoagulant protein C led to the survival of 33% and prolonged life in the fatally infected monkeys (34). This treatment was associated with decreased concentrations of D-dimers, decreased tissue factor activity, fewer fibrin thrombi, and fewer animals with increased MCP-1 levels. The survivors did not develop increased plasma concentrations of IL-6 or MCP-1. Recombinant nematode anticoagulant protein inhibits the activated factor VII–tissue factor complex. The beneficial effect was associated with attenuation of the coagulopathy and reduced fibrinolysis, fibrin deposition, and systemic inflammatory response syndrome.

**Immune Mechanisms**

Survival of filoviral infection is associated with an antibody response that is directed against
NP and VP40, \(b\) cell-mediated immunity to GP antigen, and \(c\) clearance of the virus in association with activation of cytotoxic T lymphocytes. A fatal outcome was associated with greater concentrations of proinflammatory cytokines and chemokines that appeared to be pathological in one study (19).

Filoviral infection of dendritic cells and macrophages leads to impaired T cell priming. Ebola virus infection of dendritic cells inhibits dendritic cell maturation and cytokine production, resulting in reduced T cell proliferation. Ebola virus VP35 inhibits maturation of mouse dendritic cells; induction of IL-6, IL-12, TNF-\(\alpha\), and IFN-\(\alpha/\beta\); and activation of CD4 T cells (35). Presumably, these events, along with apoptosis of T cells and NK cells, play a role in the impaired protective immune response in fatal cases.

**FLAVIVIRUSES**

Yellow fever is the original HF; it was described before the knowledge of the nature of a virus. Among the flaviviruses associated with VHF (Table 2), yellow fever virus is the first recognized etiologic agent of this syndrome. Currently, the most notorious flavivirus that causes HF is dengue virus.

**Agent**

Yellow fever virus is a 40–60-nm, single-stranded, positive-sense RNA virus. The 10.9-kb genome encodes three structural protein genes [capsid, premembrane, and envelope (\(E\))] and seven nonstructural (NS) genes (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). They are translated into a single polyprotein that is cleaved by a serine protease in the NS2B-NS3 enzyme complex into 10 proteins. The virus has a bilayer envelope of host cell origin; embedded dimers of the envelope GP build spikes on the surface. The monoclonal (M) protein also resides in the viral envelope.

**Clinical Manifestations**

Only approximately one-third of people who are infected with yellow fever virus become ill. After an incubation period of 3 to 6 days, the onset of fever and headache is abrupt. Of these patients, approximately 80% recover without suffering classic yellow fever. The remaining 20% pass through three clinical stages: infection, remission, and intoxication. During the 3–4-day period of infection, viremia appears, peaks, and then falls rapidly (36). The patients manifest fever of 39°C or higher, myalgia, nausea, vomiting, bradycardia that is relative to the level of fever, and conjunctival congestion. Marked albuminuria may be present in severe cases (36).

A period of remission is characterized by amelioration of the symptoms for up to two days. The period of intoxication usually begins 3–6 days after the onset of illness. Viremia is cleared, and IgM-neutralizing antibodies are produced. Fever, nausea, and vomiting reappear, and the patient develops jaundice, hemorrhages, and renal failure. Icterus increases sharply, reaching a peak an average of 6 days after onset; serum bilirubin concentration falls relatively rapidly after day 7. Hemorrhagic manifestations include hematemesis that is red early on, then black and chocolate colored; hematuria; epistaxis; menometrorrhagia; bleeding gums; and petechiae and ecchymoses of skin, mucous membranes, and serosal surfaces. Renal involvement occurs with increasing albuminuria on days 3 and 4 in severe cases (36). Proteinuria can reach nephrotic syndrome levels. Fatal cases manifest terminal severe oliguria or anuria and elevated serum urea and creatinine concentrations. Severely ill patients may develop agitation, delirium, seizures, stupor, and coma. The terminal stage is characterized by hypotensive shock, hemorrhages, and metabolic acidosis. The case-fatality ratio for classic yellow fever is 20%. Death usually occurs between days 6 and 8 of illness.

**Epidemiology**

Yellow fever virus is transmitted by mosquitoes in tropical Africa and South America. Zoonotic cycles involving sylvatic mosquitoes and nonhuman primates occur in tropical forests and on the edge of the African savanna. People whose
work exposes them to these mosquitoes can become infected. Yellow fever virus–infected persons can bring the virus into an urban area, where abundant *Aedes aegypti* mosquitoes then transmit the virus from person to person, which leads to an epidemic. Yellow fever virus is also maintained vertically in mosquito populations by transovarian transmission.

**Interaction Between Viral Proteins and Host Cells**

Yellow fever viral protein E attaches to host cell receptors leading to endocytosis by clathrin-coated pits and internalization into the cell. Rearrangement of the E protein exposes its fusion component, which causes fusion between the viral envelope and the cell membrane and entry of the viral genome and replicase complex into the cytoplasm. NS5 is a high-fidelity RNA-dependent RNA polymerase. Mature viral particles accumulate in the rough endoplasmic reticulum and are released by exocytosis or cell rupture.

**Human Pathology and Pathogenesis**

The hepatic lesions in fatal yellow fever are apoptosis and necrosis of hepatocytes and Kupffer cells, involving mainly the midzone of the lobule and sparing the cells adjacent to the portal triad and the terminal hepatic vein; microvesicular steatosis of the midzone and adjacent hepatocytes; decreased glycogen stores; minimal cellular inflammatory response; and retention of the reticulin structure (37, 38). In fatal cases, a median of 70% of hepatocytes are apoptotic or necrotic. Patients who die on day 8 of illness reveal mitoses that suggest that regeneration and resolution occur in survivors. Viral nucleic acids are observed in hepatocytes, occasional sinusoidal lining cells, and venous endothelium (39). The infected midzone is infiltrated more by CD4 than CD8 T lymphocytes, NK cells, antigen-presenting cells, macrophages, and B cells (38) in the setting of a quantitatively weak inflammatory cell response. FasL is increased mainly in midzonal hepatocytes. Likely events are viral activation of CD8 cytotoxic T lymphocytes that bind via Fas/FasL to infected hepatocytes, thereby inducing apoptosis of these cells (40).

The kidneys are swollen with cell death and microvesicular steatosis of tubular epithelial cells and minimal inflammatory response. The relative importance of decreased renal perfusion associated with hypotensive shock and virus-induced injury to infected tubular epithelial cells is unclear, and the pathogenesis of proteinuria is unknown. Nearly all patients who develop anuria die (41).

The pathogenesis of the hemorrhagic manifestations is better understood in yellow fever than in the other VHFIs. Hemorrhage is a grave sign. In one study, 84% of patients with melena, 67% with bleeding gums, and 79% with any hemorrhage died (41). Only 21% of the nonfatal cases developed hemorrhage. The prothrombin time is more prolonged in fatal cases, and severely prolonged prothrombin time is observed only in these cases (42). Plasma levels of coagulation factors II, V, VII, IX, and X are decreased (43). The principal cause of bleeding is decreased hepatic synthesis of clotting factors. The plasma fibrinogen concentration remains at a normal level. Central nervous system (CNS) manifestations such as extreme restlessness and terminal coma are probably caused by hypoglycemia, metabolic acidosis, and hypoperfusion. Inflammatory lesions are not present in the brain, and the brain is rarely invaded by yellow fever virus (43). Accordingly, intracerebral inoculation of nonhuman primates with yellow fever virus causes typical hepatic and other visceral pathology.

**Animal Models**

Investigators have developed nonhuman primate and hamster models of yellow fever. Disease in rhesus and cynomolgus macaques follows a fulminant course that represents the telescoped period of intoxication of human yellow fever without the other two preceding stages. The animals develop severe hepatic necrosis, jaundice, decreased levels of
coagulation factors synthesized in the liver, re-
nal tubular necrosis, hyperkalemia, and shock (44). Normal hepatic and renal histology, without scarring, are restored in the survivors (45).

A hamster-adapted yellow fever virus also provides a model characterized by viral replication in liver, kidneys, spleen, and heart; peak viremia on day 3; and death on days 6–10 (46). Pathologic lesions include hepatocellular steatosis and apoptosis followed by regeneration in survivors. There is marked lymphocyte depletion in the spleen.

**Viral Entry, Targets, and Spread**

Other than introduction of the virus by a feeding mosquito; hematogenous spread; and targeting of Kupffer cells, hepatocytes, renal tubular epithelium, and other cells, the pathogenic sequence is speculative and is based on concepts derived from other flaviviral infections.

**Pathogenic Mechanisms**

Pathology in cells infected with yellow fever virus is probably mediated by both direct viral injury and host-mediated mechanisms (e.g., cytotoxic CD 8 T lymphocyte–induced apoptosis of infected hepatocytes). The serum concentrations of cytokines and chemokines (IL-6, TNF-α, MCP-1, IP-10, and IL-1RA) are greater in fatal human cases of yellow fever than in nonfatal, nonhemorrhagic patients (47). The viremia levels are also greater in fatal cases than in nonfatal cases. Thus, the pathogenesis reflects poor immune control of infection as well as extensive hepatic necrosis and an inflammatory cascade that lead to death.

**Mechanisms of Immunity**

Immunization with the live attenuated 17D yellow fever virus vaccine or survival of naturally acquired infection confers long-lasting immunity. Neutralizing antibodies that prevent the viral E protein from attaching to the host cell receptor are thought to be the critical mechanism of protective immunity. However, antibodies to the NS1 protein, which is expressed in the host cell cytoplasm and cell surface, are also protective. Vaccination in humans stimulates the production of cytokines and CD8 T cells. The vaccine virus activates dendritic cells via Toll-like receptor 2 (TLR2), TLR7, TLR8, and TLR9 to produce proinflammatory cytokines. Innate immunity appears to be effective in subclinically infected persons.

**DENGUE HEMORRHAGIC FEVER**

**Agent**

There are four genetically and antigenically distinct dengue viruses, which are named dengue virus 1, 2, 3, and 4. Like yellow fever virus and other flaviviruses (Table 2), dengue viruses are small (40–65 nm) and spherical; they possess a lipid envelope and an 11-kb positive-sense RNA genome that encodes three structural proteins and seven NS proteins.

**Clinical Manifestations**

Most primary dengue virus infections that occur in childhood are asymptomatic (48). Acute dengue fever lasts for 2 to 7 days and is characterized by a sudden onset of fever, myalgia, severe retroorbital headache, nausea, vomiting, conjunctival congestion, and a macular rash followed by a maculopapular rash and generalized lymphadenopathy (49). In most patients, the signs and symptoms resolve, but convalescence may be prolonged. Laboratory abnormalities include neutropenia, lymphocytosis, thrombocytopenia, and elevated concentrations of hepatic transaminases. Mild to severe hemorrhages may occur.

In a small fraction of cases between 24 h before and 24 h after defervescence, plasma leakage and increased hemorrhages develop. Plasma loss leads to hypovolemia and hemo-concentration (50). The plasma leakage resolves rapidly over a 48-h period. Patients with the most severe dengue infections, known as dengue shock syndrome, develop shock, defined as a pulse pressure of less than 20 mm Hg.
Epidemiology

Dengue fever cases have increased 30-fold since the 1960s (51). Infections transmitted by A. aegypti mosquitoes have spread from Southeast Asia to India, Pakistan; islands in the Indian and Pacific oceans; East and West Africa; the horn of Africa; the Arabian peninsula; and the American tropics and subtropics, from South Texas, the Florida Keys, and the Caribbean islands to Argentina. Dengue HF (DHF) occurs at least 10-fold more frequently in infections with a second serotype of dengue virus than during primary dengue fever. Infants of mothers who have previously had dengue virus infection are at high risk of developing DHF when infected with this virus.

Interaction Between Viral Proteins and Cells

Dengue virus binds to an unknown host cell receptor by domain III of the E glycoprotein. The virus then enters the cell in an endocytic vacuole that undergoes acidification, which causes rearrangement of the E protein, fusion of the viral envelope membrane and the host’s vacuolar membrane, and movement of the viral RNA into the cytoplasm. Viral proteins are translated and assembled with replicated genomic RNA in the endoplasmic reticulum. Mature virions are produced after furin cleavage of the viral pre-M protein, which is required for budding of the infectious progeny.

The protein NS1, which is not incorporated into the viral particle, is expressed on the host cell membrane and secreted. NS3 has helicase and protease activity; its serine protease activity also requires NS2B. NS2A, NS2B, and NS4B inhibit type I IFN signaling, and NS5 is an S-adenosine methyltransferase and RNA-dependent polymerase.

Human Pathology and Pathogenesis

Most cases of DHF are caused by a second dengue virus infection, and antibodies to the first infection that do not neutralize viral infectivity can cause (a) increased entry into mononuclear phagocytes by Fc receptor-mediated endocytosis of virus and (b) subsequent increased viral replication. Although this theory better describes the greater incidence of DHF in second dengue virus infections than in initial infections and in infants who apparently received transplacental maternal antibodies, DHF can occur in primary infections, and some dengue virus strains exhibit greater virulence than others do (52).

In vitro assays of antibody-dependent enhancement of viral proliferation do not reliably correlate with disease severity, as expected, and a large portion of sera exhibits both neutralizing and enhancing activity (53). Transplacentally acquired neutralizing antibody is protective early in infancy (54). Symptomatic dengue occurs between the ages of 4 and 8 months, but there is no correlation between the occurrence of DHF and in vitro measurements of antibody-dependent enhancement.

The B cell response following primary dengue virus infection is directed predominantly against serotype-specific antigen of E protein (55). Following a second dengue virus infection, the B cell response is predominantly serotype cross-reactive, and the heterologous antibody has greater avidity than the homologous antibody does. Presumably, these characteristics of the B cell response play a role in the antibody-dependent enhancement of viral replication.

Furthermore, the magnitude of the T cell response to dengue viral antigens is directly related to the severity of disease (56). In DHF caused by the primary infection, there are greater quantities of T cells producing TNF-α and IFN-γ than in ordinary dengue fever, and the magnitude of TNF-α and IFN-γ produced by T cells correlates with the severity of disease. Patients with DHF have fewer CD4+CD8+ T cells and more cytokine-producing T cells, which suggests that these patients have a deficiency in cytotoxic T cell viral control and cytokine-mediated tissue injury in DHF.

The primary target cells are mononuclear phagocytes, including macrophages, Kupffer...
cells, and probably dendritic cells. In fatal cases, hepatocytes are infected. Pleural, peritoneal, and/or pericardial effusions are observed in three-quarters of fatal cases (57, 58). Hemorrhages involve the gastrointestinal mucosa, skin, pulmonary alveoli, and serosal surfaces (57, 59). Liver necrosis occurs frequently and typically is in a paracentral location with minimal inflammatory response (57–59). Hepatocytes often manifest microvesicular steatosis as well as apoptosis (14). Interstitial pneumonia has also been reported (58).

Interactions among the virus, the immune response, and the endothelium cause the acute plasma leakage in DHF. Before and during the leakage, the plasma concentrations of IFN-α, IFN-γ, IL-6, IL-8, IL-10, CXCL9, CXCL10, CXCL11, TNF-α, MIF, vascular endothelial growth factor (VEGF), sCD4, sCD8, TNF receptor, sIL-2R, IL-1RA, TGF-β, and activated T lymphocytes are increased. The concentrations of these elements are correlated directly with the severity of clinical illness. TNF-α is produced by dengue viral antigen–stimulated T cells only from children hospitalized with a second dengue virus infection, not from children with dengue fever who do not require hospitalization (51).

Comparison between the complement levels of patients infected with dengue virus 3 who had uncomplicated dengue fever or DHF revealed that patients with the latter disease had decreased plasma concentrations of C3 and factor H and increased levels of C3a, C4a, C5a, mannose-binding ligand, and factor D (60). Concentrations of C3a and C5a precede plasma leakage, which suggests that they play a causative role. The combination of alterations in the alternative complement pathway (increased factor D and decreased factor H) favors increased C3 convertase activity. The consumption of C3 in DHF involves both the classical and alternative pathways and correlates with the clinical severity and decreased platelet counts.

The plasma concentration of free, but not total, VEGF-A, the most potent permeability-enhancing cytokine, is increased in patients with DHF at the time of plasma leakage (61). At this point in the clinical course, the concentrations of sVEGF receptor 2 (sVEGFR2) and VEGF-VEGFR2 complex are decreased. The severity of plasma leakage is related to the concentration of sVEGFR2, which is decreased to a greater extent in patients with higher viral loads. Because sVEGFR2 is much more abundant than sVEGFR1, a low concentration of sVEGFR2 is likely to result in a higher level of free VEGF-A, as is observed in DHF. VEGF binds to VEGFR2 on endothelial cells, which causes increased vascular permeability. Thus, the lower the concentration of sVEGFR2 is, the greater the volume of pleural fluid observed. The time course fits the importance of VEGF in plasma leakage. The high viral load is followed 3 days later by decreased sVEGFR2 and plasma leakage, and the highest concentration of VEGF occurs on the day of defervescence, when plasma leakage typically occurs.

Microarray analysis of gene expression in peripheral blood mononuclear cells 2 to 3 days before plasma leakage suggests a specific pattern of gene regulation during dengue viral infection (60). There appears to be a particular pattern of gene expression in DHF that is characterized by (a) downregulated expression of immune response genes, such as complement factor D and MYD88, defense-response genes, such as the type I IFN effectors and 5′-oligoadenylate synthetases 1 and 2; and (b) upregulated expression of apoptosis-response genes. Comparison among the expression patterns of classic dengue fever, DHF, and cases classified as intermediate suggests that dengue fever and DHF lie at the extremes of a continuum.

Animal Models
The animal models of dengue virus infection employ nonhuman primates, mice lacking receptors for IFN-α/β and IFN-γ, immunodeficient mice reconstituted with human hematopoietic stem cells, and miniature swine (62). Most studies have reported that nonhuman primates inoculated with dengue virus develop neither dengue fever nor DHF;
rather, they undergo subclinical infection with viremia. However, a recent study of rhesus monkeys inoculated intravenously with $10^7$ plaque-forming units (pfu) of dengue virus 2 developed petechiae and mild to extensive subcutaneous bleeding on days 3 to 5, as well as increased plasma levels of D-dimers, thrombin-antithrombin complexes, hepatic enzymes, and creatine phosphokinase (63). The animals did not manifest fever, loss of appetite, or lethargy, and they had normal prothrombin and activated partial thromboplastin times. The viremia levels were substantially lower than those in human DHF. This incompletely characterized model does not represent severe dengue fever.

Immunocompetent mice inoculated with dengue virus develop neither disease nor viremia. IFN-α/β- and γ receptor–deficient mice infected with mouse-adapted dengue virus develop CNS infection and paralysis, the latter of which is not usually a component of human dengue fever (64). After 10 cycles of passage of dengue virus, alternating between IFN receptor knockout mice and mosquito cells, infection with $10^7$ pfu causes a short lethal course. The mice develop thrombocytopenia and plasma leakage in the intestines, liver, and spleen but not in the lungs, brain, or body cavities. Microscopic illustrations did not reveal the lesions characteristic of fatal human dengue infection.

In another report, infection of IFN-α/β- and γ receptor–deficient mice with non-mouse-adapted dengue virus 2 yielded dose-dependent responses. Infection with $10^3$ pfu was uniformly lethal; peak viremia was $10^4$ pfu ml$^{-1}$ at the time of death on day 5, and there was severe vascular leakage of Evans blue bound to plasma albumin. Infection with $10^4$ pfu of virus resulted in peak viremia of $10^5$ pfu ml$^{-1}$ on day 6, viral clearance on day 8, death on days 15 to 20, and plasma leakage terminally. Both viral doses resulted in increased plasma levels of TNF-α, IL-6, and IFN-γ at peak viremia; the levels returned to baseline in the mice that received the lower inoculum. This more promising model is inadequately characterized, and the histologic findings are not the same as those from human dengue virus infection (65).

Mice with various types of immunodeficiencies that have immune reconstitution by transfer of human CD34$^+$ hematopoietic stem cells develop viremia, fever, thrombocytopenia, and erythema; some but not all animals develop antibodies to dengue virus (66, 67). Miniature swine inoculated subcutaneously with dengue virus 1 developed viremia and, subsequently, antibodies to the virus. Reinfection caused a rash and cutaneous edema (62).

Viral Entry, Targets, and Spread

Dengue virus is inoculated into the skin through the bite of an A. aegypti mosquito. A hypothetical sequence is as follows: Initial infection of dermal dendritic cells spreads via the lymphatic vessels to the draining lymph nodes; replicates in mononuclear phagocytes; disseminates hematogenously; and replicates in Kupffer cells, other macrophages, and hepatocytes.

Pathogenic Mechanisms

Prospective human studies have elucidated more about the pathogenesis of dengue virus infections than have the limited animal and cell-culture models. Rhesus monkeys with weakly cross-reactive antibodies to the experimentally inoculated dengue virus develop higher levels of viremia that are Fc dependent, but these monkeys do not develop DHF.

Rhesus monkeys transfused with cord-blood serum containing a very low titer of neutralizing antibodies to a challenge dengue virus develop more severe viremia than do monkeys receiving nonimmune cord-blood serum. Thus, enhancement can occur in the presence of insufficient neutralization, as has been observed in children whose maternal antibody has been catabolized (68).

IFN-α/β- and γ receptor–deficient mice that are treated with antibody to TNF-α and infected with dengue virus survive past the usual period when death occurs (days 3–5 in sham-treated dengue virus–infected mice) (64). Sequential infection with different dengue virus serotypes and passive transfer of antibodies to a...
heterologous serotype have yielded results that vary from cross-protection to enhanced disease (62, 69, 70). These data support roles for low-reactivity, weak-avidity antibodies and TNF-α, but they have not improved our knowledge about the pathogenic mechanisms.

Dengue virus infection of human endothelial cell cultures causes increased production of cell-surface VEGFR2. This finding is consistent with the hypothesis that high concentrations of VEGF-A in DHF bind to VEGFR2 on endothelial cells, which leads to increased microvascular permeability (71).

MIF, which is stored in lipid droplets in human macrophages, mediates proinflammatory events involving TLR expression, cytokines, and chemokines, and manifests increased plasma levels in DHF. MIF is secreted by human macrophages infected in vitro by dengue virus in association with TNF-α, IL-6, and PGE2 (prostaglandin E2) (72). MIF-deficient mice infected with dengue virus 2 die later; have a lower viral load; have less hemocoagulation; and have no elevated IFN-γ, IL-6, and lung neutrophils and chemokines. These data support roles for all of these elements as effectors of severe dengue viral infection.

**Mechanisms of Immunity**

Humans produce antibodies to the dengue viral E, pre-M, and NS1 proteins. Domain III of the E glycoprotein, which has the most variable amino acid sequences, is the probable receptor-binding region. Neutralizing antibodies to the E protein regions inhibit receptor binding or viral envelope fusion with the host endocytic membrane. Complement fixation via antibody to the E or pre-M protein inhibits infection. Passive transfer of antibodies to the pre-M, E, or NS1 protein of the challenge dengue virus serotype protects mice from lethal disease.

T cell responses in a second dengue viral infection are quite cross-reactive. Subclinical second dengue virus infections are associated with stronger preinfection cell-mediated immune responses to dengue virus; these patients have many more dengue virus-specific T lymphocytes that produce TNF-α, IFN-γ, and IL-2. The CD4 and CD8 T cell responses are directed mostly against NS1 epitopes.

Studies of the gene responses in peripheral-blood mononuclear cells of patients with classic dengue fever or DHF that were performed 3 to 5 days after the onset of illness revealed that in the DHF patients, TLR7 and MYD88 were upregulated; these genes were downregulated in the classic dengue patients. This differential gene expression in DHF suggests that the innate immune response differs in these patients. Although a second dengue virus infection is associated with an increased likelihood of DHF, the third and fourth dengue infections are not.

**ARENAVIRUSES**

**Introduction**

Arenaviral infections are very frequent causes of acute diseases in humans. Arenaviruses cause chronic infections of rodents and occur worldwide. Infected rodents move freely in their natural habitats and may invade human dwellings. Humans are probably infected through mucosal exposure, through aerosols, or by direct contact of abraded skin with infectious materials. Some of the (re)emerging arenaviruses cause severe HF in human patients with potentially lethal outcome. For example, Junin virus causes Argentine HF (AHF), a disease endemic to the pampas region of Argentina with an at-risk population of approximately five million people (73, 74).

In addition, increased travel to and from endemic regions where pathogenic arenaviruses circulate may import VHF into nonendemic areas. Tourists from the United States or western Europe are diagnosed with Lassa fever upon return from travel in Africa. In addition to having an impact on public health, some of these viruses, such as Junin virus, possess features that could make them biological weapons. For example, Junin viruses are very stable and highly infectious by aerosol, and they cause high morbidity and significant mortality at low doses.

**AHF: Argentine hemorrhagic fever**
AHF was first described in 1953, and the virus was isolated several years later. A similar infectious disease was reported in Bolivia in 1959, and the causative agent, Machupo virus, was first isolated in 1965. In 1989, an outbreak of HF occurred among a rural population in Venezuela, and the agent, Guanarito virus (GTOV), was identified. The most recent agent of South American VHF, Sabia virus, was isolated in 1990 in Brazil (75). All of these viruses belong to the group of New World arenaviruses in the family Arenaviridae.

Lassa fever is an acute, systemic infectious disease that was first described in the town of Lassa, Nigeria, in 1969. This severe human disease is caused by the most important Old World arenavirus, Lassa virus (LASV) (76).

Agents
Arenaviruses are enveloped viruses with a bisegmented negative-strand RNA genome (77). Each of the two genomic RNA segments, L (∼7.3 kb) and S (∼3.5 kb), uses an ambisense coding strategy to direct the synthesis of two polypeptides in opposite orientations; these polypeptides are separated by a noncoding intergenic region that acts as a transcription termination signal for the virus polymerase (78, 79). The S RNA segment encodes the viral glycoprotein precursor (GPC) and the nucleoprotein. GPC is posttranslationally cleaved by the cellular site 1 protease to yield two glycoproteins, GP1 and GP2, which are embedded in the lipid bilayer to form the viral spikes in the mature virion that are crucial for receptor recognition and virus entry (80–82). The L RNA segment encodes the viral RNA-dependent RNA polymerase (83) and the small (∼11-kDa) RING finger protein Z, which is the arenavirus counterpart of the matrix protein found in many other negative-strand RNA viruses (84–86).

Clinical Manifestations
The South American VHF's resemble one another, and AHF is the best characterized. The onset of the clinical disease is similar to those of many infectious diseases; it is characterized by malaise, anorexia, chills, headache, myalgias, and fever (38°C to 39°C). A few days after onset, patients may develop constitutional, gastrointestinal, cardiovascular, and neurological signs and symptoms. Low backache, retroorbital pain, nausea, vomiting, epigastric pain, photophobia, dizziness, and constipation or mild diarrhea have been reported. The absence of productive cough or nasal congestion is helpful in distinguishing the initial symptoms of AHF from those of influenza or other acute respiratory infections.

In the respective endemic areas, or in patients with a history of travel to the specific geographic regions, a febrile syndrome with proteinuria, leukopenia, and thrombocytopenia raises suspicion of one of the South American VHFs (87–89). Among the systemic infectious diseases, the differential diagnosis includes typhoid fever, hepatitis, infectious mononucleosis, leptospirosis, hantavirus pulmonary syndrome, dengue, DHF, and rickettsioses. Malaria should also be considered in endemic areas. The definitive diagnosis depends on demonstration of the infecting virus or one of its products in acute serum.

Reverse-transcription polymerase chain reaction is usually the most sensitive diagnostic assay and produces amplicons that can be sequenced for genetic analysis. In general, viremia and antigenemia are readily detected during the acute phase and disappear as the patient recovers. The presence of viral nucleic acid can be detected during the same period and sometimes 1 or 2 days longer (87).

Seroconversion, mainly IgM antibodies, may be detected during illness and usually appear early in convalescence. Diagnosis of initial patients in any outbreak, particularly if there are unusual features, benefits from the study of virus isolates and classic serology. Most of the HF viruses are hazardous and should be isolated or studied only under BSL-4 (biosafety level four) containment. Blood samples from patients should be collected early in the course of illness, and both serum and blood clot...
samples should be frozen as soon as possible for potential virus isolation. A second serum sample should be obtained for comparative serology. In fatal cases, a full autopsy should be performed with a complete set of organs collected in formalin for diagnostic studies; spleen, liver, and lymph nodes should be frozen unfixed for virus isolation. Classic histopathology is often useful in raising the possible diagnosis of yellow fever, Rift Valley fever, or a filovirus infection, as well as in diagnosing some of the confounding diseases. Immunohistochemistry on fixed tissues can usually establish a definitive diagnosis.

**Epidemiology**

Arenaviruses cause chronic infections in rodents and have a wide regional distribution. Infected rodents move freely in their natural habitats and can invade houses. Humans are infected through mucosal exposure, through aerosols, or by direct contact of abraded skin with infectious material. Person-to-person transmission is very rare and may occur via direct contact with infected body fluids of a viremic patient. Nosocomial infections have been reported.

**Lassa fever.** Epidemics of Lassa fever occur in parts of West Africa, including Liberia, Guinea, Sierra Leone, Nigeria, and the Central African Republic. The peak incidence is between January and April, and the disease affects all age groups and both sexes. However, serological studies from Mali, Senegal, and the Democratic Republic of Congo indicate that the distribution may be even wider.

LASV causes lifelong infection in rodents of *Mastomys* species (multimammate rats) that are distributed over large parts of Africa. Although the virus causes no apparent disease in the natural host, viral replication in rodents leads to infection of various organs and subsequent shedding via urine and feces.

**Argentine hemorrhagic fever.** Natural outbreaks occur predominantly during the major harvest season; the peak incidence is in the month of May. The disease is four times more prevalent in males than in females and is more prevalent among rural workers than in urban populations.

**Bolivian hemorrhagic fever.** The reservoir for the causative pathogen of Bolivian HF, Machupo virus, is a species of the genus *Calomys*. This rodent lives in habitats where grassland intergrades into forest and, unlike *C. musculus*, thrives in villages and near human habitations. Bolivian HF tends to be a seasonal disease; more cases occur in the dry season at the peak of agricultural activity. Epidemics in towns may occur during epizootic conditions when rodent densities reach unusually high levels and rodents invade towns or villages. In 1963–1964, a Bolivian HF epidemic occurred in the village of San Joaquin; there were 637 cases of illness and 113 deaths among the town’s 3,000 residents.

Transmission to humans under these conditions is probably caused by inhalation of infectious aerosols; however, direct contact of broken skin or mucous membranes with rodent excreta or contaminated fomites such as food may also be responsible. Person-to-person transmission, probably via direct contact with infectious body fluids, may occur in familiar or nosocomial settings. Transmission by intimate contact during convalescence has also been observed.

**Venezuelan hemorrhagic fever.** In 1989 in Venezuela, an outbreak of severe HF was reported in Guanarito, Guanarito State; the causative agent, GTOV, was soon isolated. This particular pathogen is specifically associated with *Zygodontomys brevicauda*, whereas a second arenavirus, Pirital virus, is associated with sympatric *Sigmodon alstoni*. Most epidemics occur from November to January, which coincides with increased agricultural activity. As in AHF, the highest risk is among adult male agricultural workers. Thus, transmission to humans is probably related to agricultural
activities (96, 97). Person-to-person transmission or nosocomial infection has not been observed in Venezuelan HF patients.

**Human Pathology**

Arenaviruses cause systemic infectious diseases in humans that are often severe and potentially lethal, while inducing mild pathological changes.

**Lassa fever.** Patients who succumb to LASV infection generate very limited anti-LASV immune responses, or no response at all, and histological examination of Lassa fever cases has revealed minimal immune cell infiltrates and tissue damage (91, 98). These findings support the hypothesis that morbidity and mortality associated with LASV infection are facilitated by the failure of the host’s innate defense mechanisms to limit virus multiplication early during infection and to initiate an effective adaptive immune response that can control and eliminate the infection. Accordingly, the viremia level is a good predictor of the disease outcome for Lassa fever patients (89, 99). However, few histological findings have been reported, except for hepatic necrosis and mononuclear phagocytic activation, splenic necrosis in the marginal zone of the periarteriolar lymphocytic sheath, and cytoplasmic inclusions in cells near the junction of the zona reticularis of the adrenal glands. Lesions in other organs are infrequent and very mild; they are characterized by rare myocarditis, pneumonitis, gastrointestinal mucosal petechiae, and interstitial nephritis. Importantly, no intravascular fibrin thrombi are observed, which indicates that intravascular coagulation does not occur (98).

**South American hemorrhagic fever.** Usually, hemorrhages occur in many organs, and effusions appear in serous cavities. These findings are in contrast with those from Lassa fever cases in humans, where hemorrhage is seldom present. However, hemorrhage may be also absent in some patients with South American VHF. In general, there is widespread necrosis, which may be present in any organ and varies from modest and focal to massive and multifocal. Liver and lymphoid systems are usually extensively involved, and the lung regularly has varying degrees of interstitial pneumonitis, diffuse alveolar damage, and hemorrhage. The typical inflammatory response in many viral diseases is usually minimal.

AHF during pregnancy is uncommon, but more than half of AHF patients in the last trimester die, at least in part because of tardy recognition of the disease and failure to administer specific treatment. Congenital malformations and fetal and neonatal deaths also occur (87, 100). Children tend to have a milder clinical course, but severe and even fatal disease has been reported.

Arenaviruses cause chronic infection in the natural rodent host. However, humans and some experimental animals infected with pathogenic arenaviruses often develop severe acute disease with a potentially lethal outcome. The infection is often disseminated and involves various organs (87). The visible damage to the organs is minor and is associated with minor infiltrations by inflammatory cells. It is still unclear how the virus triggers the multiorgan failure that can lead to death and why some patients develop hemorrhages while others do not.

**Animal Models**

Several experimental animal models exist for the study of arenavirus-mediated pathogenesis, given that multiple rodent species—C3H and athymic mice (101–104), guinea pigs (105–107), and rats (108), as well as New and Old World primates (109–113) [Cebus paella, Callithrix jacchus, and Macaca mulatta (110–114)]—are susceptible to infection. However, the pathogenesis (such as the median lethal dose, timing of death, and the viral distribution in organs) varies widely, depending on the animal species, virus strain, and/or passage history (105, 111, 114), and is age dependent (109, 115, 116). For example, guinea pigs and nonhuman
primates, when infected via the intraperitoneal and aerosol routes, respectively, most closely manifest a disease syndrome similar to AHF (105, 111, 117–120); these animals have been used for the evaluation of antiviral drugs as well as Junin virus vaccine candidates (121–124). They have also been successfully used in studies with LASV, GTOV, and the Machupo virus.

Outbred guinea pigs represent a good model for AHF because they reproduce most of the clinical signs as well as the pathological lesions of this disease (Figures 1 and 2). For example, infection with Junin virus causes aspartate transaminase elevation similar to that reported in human patients. Pathologic manifestations have been reported in the spleen and liver of infected guinea pigs (106). The white pulp of the spleen appears moth-eaten; there are numerous tingible body macrophages and large, reactive pale cells suggestive of macrophages. This feature involved up to 50% of the white pulp in all the animals examined in this study. The red pulp showed generalized cellular depletion, along with smudgy or fibrinoid necrosis and scattered nuclear debris. Liver pathology in ill animals is characterized by the presence of diffuse microvesicular steatosis, mild portal inflammation, and mild lobular inflammation (106).

There are many similarities among the various arenaviruses and among the diseases they cause. However, in general, the fewest pathological manifestations are present in Lassa fever.

**Viral Entry, Targets, and Spread**

To establish productive infection in the host, the virus must be able to enter a permissive cell and initiate the production of infectious progeny that, upon release, disseminate within the organism and carry the infection to various tissues.

**Portal of entry.** Arenaviruses enter the host via the mucosal membrane, cutaneous wounds, or aerosol and enter host cells by attaching onto a cellular receptor, which leads to endosomal uptake, fusion of the viral envelope and host cell membrane of the late endosome, and release of the viral contents into the cytoplasm. Human transferrin receptor 1 is the likely cellular receptor for clade B New World arenaviruses (125). The identification of transferrin receptor 1 as the cellular receptor for New World arenaviruses represents a pathogenetic difference from Old World arenaviruses such as LASV, which utilize 𝛼-dystroglycan as a receptor. In addition, Junin virus enters the cell by receptor-mediated endocytosis into clathrin-coated vesicles (126). Therefore, we emphasize that the New World arenaviruses differ from Old World arenaviruses in that the former require a different cellular receptor, as well as clathrin, for endocytosis.

After humans are exposed to infectious aerosols, productive infection with arenaviruses is probably established in macrophages (119). For example, in lethal cases of AHF, macrophages are loaded with viral antigen, as has been demonstrated by immunofluorescence (127). **Target organs and spread.** Viral infection is probably established first in lung macrophages upon aerosol exposure. Soon thereafter, virus spread occurs via blood and probably lymph to nearly all major organs. However, the exact sequence of events is largely unknown.

Given that these viruses appear to have a very broad cellular tropism, they can infect many different cell types and organs. As in vitro experiments have shown, many of these viruses can efficiently infect dendritic cells, macrophages, megakaryocytes, hepatocytes, endothelial cells, renal epithelial cells, and astroglia (91, 128). However, infection in humans is often found in spleen, liver, bone marrow, heart, adrenal glands, and kidneys. Brain infection has been reported in animal models of LASV and Junin virus but not in human cases; however, patients who survive infection with Junin virus often develop neurological sequelae that may indicate CNS infection. The role of endothelium has been often discussed,
Figure 1
Histopathology and tissue viral antigen distribution in Romero-infected guinea pigs. (a) Histopathologic analysis. Tissue sections from guinea pigs that were infected with the Romero strain of the Junin virus (JUNV) or that were mock infected were subjected to standard H&E (hematoxylin and eosin) staining. Magnification, 20 × . (b) Dissemination of this strain in the brain and liver of infected guinea pigs. Tissue sections were probed with JUNV-group hyperimmune serum and secondary biotinylated antibody. Color development was performed by use of streptavidin peroxidase, followed by the addition of a chromogenic substrate (brownish red).
Immune Mechanisms

The induction of type I IFN is a critical event in the establishment of the antiviral innate immune response; it limits the spread of infection and the subsequent mobilization of adaptive immune responses. Viral RNA is recognized by cell-surface TLRs, such as TLR2, TLR3, TLR7, and TLR9, or by cytoplasmic helicases RIG-I, and/or Mda5, which are pattern recognition receptors (PRRs) referred to as RIG-I-like receptors (129, 130). However, it is still unclear which of the TLRs and/or PRRs recognize the RNA of pathogenic arenaviruses (131). Nevertheless, when activated by viral RNA, PRRs—through association with specific signaling adapter molecules—trigger transcription of cytokines and type I IFNs. The activation of IRF-3 is a critical point in the induction of the immediate early-phase IFN-β and IFN-α/4 responses (132). In an autocrine fashion, early IFN stimulates the production of the transcriptional factor IRF-7, thereby activating IFN-α2, IFN-α5, IFN-α6, and IFN-α8 through JAK-STAT signaling (132).

Because of the potent antiviral activities mediated by type I IFN, many viruses, including arenaviruses, have evolved strategies to target the RLH-MAVS (RIG-I-like helicase–mitochondrial antiviral signaling protein) pathway to evade the host antiviral response (133, 134). Blockage of IRF-3 translocation has been demonstrated with lymphocytic choriomeningitis virus, an Old World arenavirus, or through the expression of the nucleoproteins of various highly pathogenic arenaviruses, including LASV and Junin virus. Furthermore, the induction of type I IFN is inhibited in persistently infected lymphocytic choriomeningitis virus cells and in cells expressing arenavirus nucleoproteins (135, 136). However, the interaction between pathogenic arenaviruses and the IFN pathway in the context of virus-infected cells has not been well characterized. Likewise, it is unknown whether some pathogenic and nonpathogenic strains of Junin virus or other arenaviruses induce different host innate defense responses. For
example, the interaction between Junin virus and the IFN pathway in Junin virus–infected human cells provides clear experimental evidence that non-monocyte-derived and macrophage-derived human cells, such as lung epithelial cells, efficiently recognize Junin virus during the early stages of infection. This recognition leads to a potent RIG-I-dependent IFN response that nonetheless has a very limited effect on Junin virus multiplication (137).

This host-virus interaction could be of crucial importance in pathogenesis because high levels of IFN-α (2,000–64,000 U ml⁻¹) and other cytokines, such as TNF-α, are frequently present in the sera of AHF patients (138–143). High levels of IFN also coexist with high viremia and severe or fatal disease (139, 140), which would be expected if the virus induces the IFN production but is not sensitive to its antiviral activity, as has been demonstrated in in vitro and in vivo models of AHF (144). However, this is not the case in LASV infection, and high levels of IFN are usually not present; these findings point to the differences in the pathogeneses of Old World and New World arenaviruses (91).

In most arenavirus infections, the lymphoid tissue is heavily targeted; however, the inflammatory responses in this tissue are minimal (127). Lymphopenia develops, often as a consequence of the systemic infection in humans (91) as well as in animal models (106); however, there is no indication that any of arenaviruses replicate directly in lymphocytes. One of the potential mechanisms responsible for the loss of lymphocytes is apoptotic cell death induced by cytokines, as has been proposed for Junin virus. However, how progressive lymphopenia develops is unknown. LASV also affects the maturation of dendritic cells and leads to very weak activation of T cells (145). The combination of lymphopenia and reduced T cell activation has an immunosuppressive effect, allowing the virus to cause disseminated infection and to replicate to high levels. As reported in experimental models and, in some cases, in patients, the survival correlates with development of the T cell response in the case of Lassa fever; however, neutralizing antibodies are protective in the case of AHF and probably other New World HF s. For example, the passive transfer of antibody in clinical setting reduces the lethality below 1% in human patients (146, 147), and GPC-specific antibody alone is sufficient to protect guinea pigs against lethal infection with Junin virus (122). However, complement is necessary for neutralization of the virulent Junin virus and acts through the classical pathway (148). In AHF patients with severe disease, serum levels of C2, C3, and C5 are reduced, while the level of C4 is increased (149, 150).

**Mechanisms of Cell and Organ Injury**

In general, highly pathogenic arenaviruses cause very little cytopathic effect in cell-culture systems (77, 90). Accordingly, minimal necrosis and/or cell damage has been reported from lethal cases of human patients or animal models. LASV infection in vitro and in vivo is probably the best example of a “benign” cell infection; there is almost no detectable impact on cellular functions. For example, there are minimal to no transcriptional changes in human hepatoma cells infected with LASV, which indicates either that the infection is probably not recognized or that the host response is efficiently inhibited (151). However, the virus replicates to high levels and can establish persistence in vitro. This phenomenon also occurs in vivo, such as in mice that lack a functional IFN system and develop persistent infection with little to no detectable clinical disease, as well as in the natural host (152). New World arenaviruses, such as Junin and Machupo viruses, are slightly more pathogenic in cell cultures; however, they also tend toward persistent infection. An important characteristic of Junin virus infection is its ability to induce IFN production in vitro and in vivo. More recent experimental data indicate that the RIG-I/IRF-3 signaling pathway is activated in human lung epithelial cells infected with Junin virus, which causes transcriptional changes in many IFN-sensitive genes (137). However, the potential impact of these changes on cell function is largely unknown. Similar
to lung epithelial cells, human umbilical vein endothelial cells have no cytopathic effect and do not demonstrate apoptosis when infected with Junin virus. Nevertheless, this infection leads to upregulation of ICAM-1 and VCAM-1, whereas von Willebrand factor is not upregulated (128). Human umbilical vein endothelial cells also produce nitric oxide, endothelial nitric oxide synthase, prostacyclin, and decay-accelerating factor when infected with Junin virus. Some of the changes that have been observed in vitro may be important for disease development in vivo; however, more research is needed. Also, LASV causes minimal changes in infected endothelial cells (153). However, in rhesus monkeys infected with LASV the endothelium is only minimally infected, thereby causing limited necrosis (154).

Although direct viral impact is minimal, it is well established that many of these infections induce cytokine production in the host that may contribute to disease development while exerting a minor antiviral effect. A current hypothesis of an indirect effect on cell function comes from studies with Junin virus. These studies proposed that the infection and strong upregulation of IFN production directly affect megakaryocytes and indirectly affect platelet function. These biological effects would contribute to the development of thrombocytopenia and hemorrhages, as has been reported in human cases (155). In summary, arenaviruses probably cause little direct cell damage while inducing a systemic host response that may affect various organ systems, contributing to multiorgan failure, hemorrhages, and ultimately death.

**SUMMARY POINTS**

1. VHFs vary from uniformly severe infections, with case-fatality rates greater than 60% (e.g., filoviruses), to mostly asymptomatic infections, wherein a minority of infected persons develop HF (e.g., yellow fever virus and dengue virus).
2. Despite the name VHF, patients with some of these diseases seldom develop hemorrhage. In some other VHFs, the percentage of patients with bleeding is less than 50%, and in many patients with bleeding, the hemorrhages are not clinically significant.
3. The mechanisms of hemorrhage and plasma leakage in VHFs include pathologic concentrations of cytokines and other mediators, platelet aggregation and consumption, activation of the coagulation cascade, endothelial injury, and insufficient coagulation factors because of severe hepatic necrosis.
4. Pathological lesions vary from organ necrosis that reflects severe clinical illness to low levels of cell death in a few organs that do not account for the patient’s death.
5. The currently available animal models of VHFs are not ideal for determining the mechanisms of immunity and cell and tissue injury.
6. Research efforts on VHFs have ranged from extensive, highly illuminating human clinical studies, such as those of DHF; to numerous experimental studies of pathogenesis in nonhuman primates of infection with Ebola virus, which causes only sporadic outbreaks; to the remarkable neglect of Lassa fever, which causes up to 500,000 human infections and many deaths annually.

**DISCLOSURE STATEMENT**

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