Chapter 13

VIRAL HEMORRHAGIC FEVERS

PETER B. JAHRLING, PhD*; AILEEN M. MARTY, MD†; AND THOMAS W. GEISBERT, PhD‡

*Director, National Institute of Allergies and Infectious Diseases, Integrated Research Facility, National Institutes of Health, 6700A Rockledge Drive, Bethesda, Maryland 20892; formerly, Senior Research Scientist, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland

†Senior National Security Advisor, Medical Instructor, Battelle Office of Homeland Security, Battelle Memorial Institute, Suite 601, 1550 Crystal Drive, Arlington, Virginia 22202; formerly, Professor, Pathology and Emerging Infections, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland

‡Chief, Department of Viral Pathology and Ultrastructure, US Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702

INTRODUCTION

Viral hemorrhagic fever (VHF) is an acute febrile syndrome characterized by systemic involvement, which includes generalized bleeding in severe infections. Patients with VHF manifest combinations of malaise, prostration, generalized signs of increased vascular permeability, and coagulation abnormalities. Although the more severely ill patients manifest bleeding, this does not result in a life-threatening loss of blood volume. To a certain extent, however, it indicates damage to the vascular endothelium and is an index of disease severity in specific target organs. Much of the disease appears to be caused by dysregulation of the innate immune response, although replication of these hemorrhagic fever (HF) viruses in target cells and tissues can directly contribute to the pathological manifestations of VHF. Factors that may contribute to this subversion of the host immune response include the rapid infection and impairment of dendritic cells, a sudden and enigmatic death of lymphocytes, and the release of a variety of mediators from virus-infected cells that subsequently alter vascular function and trigger the coagulation disorders that epitomize these infections.

The viral agents causing severe HF, which are taxonomically diverse, are all single-stranded RNA viruses that can infect humans through contact with contaminated animal reservoirs or arthropod vectors. Under natural conditions, these viruses cause significant infectious diseases, although their geographical ranges may be tightly circumscribed. The relatively recent advent of jet travel coupled with human demographics increase the opportunity for humans to contract these infections; from time to time, sporadic cases of VHF are exported from endemic areas to new areas. Clinical and epidemiological data on VHFs are sparse; outbreaks are sporadic and unexpected, and typically develop in geographical areas where cultural customs and logistical barriers encumber systematic investigations.

Because many VHFs spread easily in hospitals to patients and staff alike, causing high morbidity and mortality, they gained public notoriety in the past decade from the enormous interest and fear generated by the news media. Ebola, an HF virus with a high case-fatality rate (near 90% in some outbreaks), dramatic clinical presentation, and lack of effective specific treatment, was highly publicized when a new Ebola species was isolated in a suburb of Washington, DC, in 1989.[1] Progress in understanding the genesis of the pathophysiological changes that make Ebola and other HF viral infections of humans so devastating has been slow, primarily because special containment is required to safely work with most of these viruses.

Many of the VHF agents are highly infectious by aerosol. Most VHF agents are also stable as respirable aerosols, which means that they satisfy at least one criterion for weaponization, and some have potential as biological terrorism and warfare threats. Most of these agents replicate in cell culture to concentrations sufficiently high to create a small terrorist weapon, one suitable for introducing lethal doses of virus into the air intake of an airplane or office building. Some replicate to higher concentrations, with obvious potential ramifications. Because the VHF agents cause serious diseases with high morbidity and mortality, their existence as endemic disease threats and as potential biological warfare weapons suggests a formidable potential impact on public health.

HISTORY AND EPIDEMIOLOGY

Natural Disease

Under natural conditions members of the *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, and *Flaviviridae* (Table 13-1) that cause VHF have specific geographic distribution and diverse modes of transmission. Although the natural reservoir for *Filoviridae* remains unknown, as a group, the HF viruses are linked to the ecology of their vectors or reservoirs, whether rodents or arthropods. These characteristics have great significance not only in the natural transmission cycle for arenaviruses and bunyaviruses (rodents to humans) and for flaviviruses (arthropods), but also in the potential for nosocomial transmission. Most reservoirs tend to be rural, and a patient's history of being in a rural locale is an important factor to consider when reaching a diagnosis. Human-to-human spread is possible for most of the HF viruses. The majority of person-to-person spread has been attributed to direct contact with infected blood and body fluids. Airborne transmission of VHF agents appears to be an infrequent event, but cannot categorically be excluded as a mode of transmission.

Arenaviridae

The name *arena* is derived from the Latin words "arenosus" (sandy) and "arena" (sand) in recognition of the sand-like ribosomal contents of virions in

TABLE 13-1

VIRAL HEMORRHAGIC FEVERS OF HUMANS

Virus Family *Genus*	Virus	Disease	Natural Distribution	Source	Incubation (Days)
Arenaviridae					
Arenavirus	Lassa	Lassa fever	West Africa	Rodent	5–16
	Junin	Argentine HF	South America	Rodent	7–14
	Machupo	Bolivian HF	South America	Rodent	9–15
	Sabia	Brazilian HF	South America	Rodent	7–14
	Guanarito	Venezuelan HF	South America	Rodent	7–14
	Whitewater Arroyo	Unnamed HF	North America	Rodent	Unknown
Bunyaviridae					
Nairovirus	Crimean-Congo HF	Crimean-Congo HF	Africa, Central Asia, Eastern Europe, Middle East	Tick	3–12
Phlebovirus	Rift Valley fever	Rift Valley fever	Africa, Saudi Arabia, Yemen	Mosquito	2–6
Hantavirus	Agents of HFRS	HFRS	Asia, Balkans, Europe*	Rodent	9–35
Filoviridae					
Ebolavirus†	Ebola	Ebola HF	Africa	Unknown	2–21
Marburgvirus	Marburg	Marburg HF	Africa	Unknown	2–14
Flaviviridae					
Flavivirus	Dengue	Dengue HF	Asia, Africa, Pacific, Americas	Mosquito	Unknown
	Yellow fever	Yellow fever	Africa, tropical Americas	Mosquito	3–6
	Omsk HF	Omsk HF	Central Asia	Tick	2–9
	Kyasanur forest disease	Kyasanur forest disease	India	Tick	2–9

HF: hemorrhagic fever; HFRS: hemorrhagic fever with renal syndrome
*The agents of hantavirus pulmonary syndrome were isolated in North America.
†There are four species of Ebola: Zaire, Sudan, Reston, and Ivory Coast.

thin section under the electron microscope. The family *Arenaviridae* contains a single genus, *Arenavirus*. However, the arenaviruses are divided into an Old World group (eg, Lassa virus) and a New World group (South American and North American HF viruses) by phylogenetic analysis of RNA and serology. The New World complex is further divided into three major clades: A, B, and C. All of the viruses causing HF belong to clade B.[2] Arenaviruses survive in nature by a lifelong association with specific rodent reservoirs. Rodents spread the virus to humans, and outbreaks can usually be related to some perturbation in the ecosystem that brings humans in contact with rodents or material contaminated by rodent products. Arenaviruses initiate infection in the nasopharyngeal mucosa.

Lassa fever made a dramatic appearance in 1969 when an American nurse working at a modest mission station in Lassa, a small town in northeastern Nigeria, became ill and started a chain of nosocomial infections that extended from healthcare workers in Africa to laboratory workers in the United States. Lassa virus produces Lassa fever, a major febrile disease of West Africa that causes 10% to 15% of adult febrile admissions to the hospital and perhaps 40% of nonsurgical deaths.[3] Lassa virus infects 100,000 to 300,000 people annually in West Africa, kills 5,000 to 10,000, and leaves approximately 30,000 deaf.[3,4] Lassa fever causes high mortality in pregnant women and is also a pediatric disease. Most Lassa virus infections are traceable to contact with the carrier rodent, the rat (*Mastomys natalensis)*, but nosocomial transmission is also possible. Lassa fever has periodically been imported to Europe, the United States, Canada, and Japan by travelers from West Africa.[5] Since 2000 at least five fatal Lassa fever cases have occurred in the United Kingdom, Germany, the Netherlands, and the United States.[6,7]

Argentine HF (AHF) was described in 1943, and Junin virus was first isolated from one of its victims in 1958. Junin virus, which is carried by field voles such as *Calomys musculinus* and *Calomys laucha*, is primarily associated with agricultural activities in the pampas of Argentina, where there have been 300 to 600 cases per year since 1955.[8] Transmission is airborne from fomites, contaminated food or water, or abrasions to the skin. Direct person-to-person transmission is rare.

In 1959 physicians at the Beni department of Bolivia noted a sporadic hemorrhagic illness in patients from rural areas, which soon became known as Bolivian HF. In 1963 Machupo virus was isolated from patients with Bolivian HF, and shortly thereafter voles (*Calomys callosus)* were identified as the rodent reservoir.[9] Machupo virus produced several outbreaks of disease in the 1960s, but more recently Bolivian HF has manifested only sporadically; there was a cluster of cases in 1994. Transmission is through contaminated food and water and direct contact through breaks in the skin; there is only rare documentation of human-to-human transmission.

In 1989 an outbreak of VHF involving several hundred patients in the municipality of Guanarito, Portuguesa state, Venezuela, led to the isolation of Guanarito virus and identification of its probable animal reservoir, the cotton rat (*Sigmodon hispidus*).[10] Sabia virus caused a fatal VHF infection in Brazil in 1990,[11] a severe laboratory infection in Brazil in 1992, and another laboratory-acquired infection in the United States in 1994. The most recently recognized arenavirus linked to VHF is Whitewater Arroyo virus, which apparently caused three fatal cases of HF in California between 1999 and 2000.[12]

Bunyaviridae

Of the five genera that comprise the family *Bunyaviridae*, three genera contain viruses that cause HF: (1) *Phlebovirus* (eg, Rift Valley fever virus); (2) *Nairovirus* (eg, Crimean-Congo HF virus); and (3) *Hantavirus* (eg, Hantaan virus). *Bunyaviridae* is transmitted by arthropods (primarily mosquitoes, ticks, and phlebotomine flies), or, as is the case for hantaviruses, by contact with rodents or rodent products. Transmission by aerosol is also documented.

The phlebovirus Rift Valley fever (RVF) virus, which causes RVF, is a significant human pathogen. Outbreaks of this major African disease often reflect unusual increases in mosquito populations.[13] RVF virus, which primarily affects domestic livestock, can cause epizootic disease in domestic animals. RVF was first described in 1931 as an enzootic hepatitis among sheep, cattle, and humans in Kenya.[14] During 1950–1951, an epizootic of RVF in Kenya resulted in the death of about 100,000 sheep. An RVF epizootic can lead to an epidemic among humans who are exposed to diseased animals. Risk factors for human infection include contact with infected blood, especially in slaughterhouses, and handling of contaminated meat during food preparation. Exposure to aerosols of RVF virus is a potential source of infection for laboratory workers. In 2000 RVF spread for the first time beyond the African continent to Saudi Arabia and Yemen, affecting both livestock and humans.[15]

Crimean-Congo HF (CCHF) is a zoonotic disease transmitted not only through the bite of at least 29 species of ticks, of which *Hyalomma marginatum* is the most important, but also by exposure to infected animals or their carcasses, contact with blood and bodily secretions of infected persons, and by aerosol. The agent of CCHF is a *Nairovirus*. Although descriptions of this illness can be traced to antiquity, this disease was first recognized in 1944–1945 when a large outbreak occurred in the Steppe region of western Crimea among Soviet troops and peasants helping with the harvest. In 1956 a similar illness was identified in a febrile child from what was then the Belgian Congo (now the Democratic Republic of the Congo), but it was not until 1969 that researchers realized that the pathogen causing Crimean HF was the same as that responsible for the illness in the Congo. The linkage of the two place names resulted in the current name for the disease and the virus. CCHF is endemic in many countries in Africa, Europe, and Asia; it causes sporadic, yet particularly severe, VHF in endemic areas.[16] CCHF is often associated with small, hospital-centered outbreaks, owing to the profuse hemorrhage and highly infective nature of this virus in humans exposed by aerosol. An HF outbreak on the Pakistani-Afghan border during the 2001–2002 US campaign against terrorists is suspected to have been caused by the CCHF virus, and various media outlets have reported that CCHF was confirmed by a laboratory in South Africa.

Hantaviruses, unlike other bunyaviruses, are not transmitted by infected arthropods; rather, contact with infected rodents and their excreta leads to most human infections. However, person-to-person transmission was described during a recent outbreak of hantavirus pulmonary syndrome (HPS) in southwest Argentina,[17] and researchers have also documented transmission by aerosol.[18] Of the more than 20 known types of hantaviruses, at least nine (Hantaan, Seoul, Puumala, Dobrava, Sin Nombre, New York, Black Creek Canal, Andes, and Bayou) hantaviruses can cause significant clinical illness. Each virus has its own rodent vector, geographic distribution, and clinical expression. The poor sanitary conditions of combat promote exposure to rodents. A

review of illness during the US Civil War, World War I, and World War II suggests that outbreaks of hantaviral infections occurred among troops. Hantaviral disease was described in Manchuria along the Amur River, and later among United Nations troops during the Korean War, where it became known as Korean HF.[19] The prototype virus from this group, Hantaan, which causes Korean HF with renal syndrome (HFRS), was isolated in 1977. The reservoir host for Hantaan virus is the striped field mouse (*Apodemus agrarius)*.

Hantaan virus is still active in Korea, Japan, and China. Seoul virus, which is carried mainly by the house rat (*Rattus norvegicus)*, causes a milder form of HFRS and may be distributed worldwide. Other hantaviruses associated with HFRS include the Puumala virus, which is associated with bank voles (*Clethrionomys glareolus*). An epidemic in 1993 in the Four Corners region of the United States led to the identification of a new hantavirus (Sin Nombre virus), and eventually to identification of several related viruses (Black Creek Canal, New York, Andes, and Bayou); all of these have been associated with HPS.[20,21] The classical features of the syndrome of acute febrile illness associated with prominent cardiopulmonary compromise have been extended to clinical variants, including disease with frank hemorrhage.[21]

Filoviridae

Marburg virus and Ebola virus, the causative agents of Marburg and Ebola HF, respectively, represent the two genera that comprise the family *Filoviridae*. The *Marburgvirus* genus contains a single species: *Lake Victoria marburgvirus*. The *Ebolavirus* genus is divided into four distinct species: (1) *Ivory Coast ebolavirus*, (2) *Reston ebolavirus*, (3) *Sudan ebolavirus*, and (4) *Zaire ebolavirus*. By electron microscopy, filoviruses have a highly unusual filamentous appearance. The term filovirus was derived from "filo," which is Latin for thread. Marburg virus was first recognized in 1967 when three simultaneous outbreaks of a lethal VHF epidemic occurred at Marburg and Frankfurt, Germany, and Belgrade, Yugoslavia, among laboratory workers exposed to the blood and tissues of African green monkeys (*Chlorocebus aethiops*) imported from Uganda. Secondary transmission to medical personnel and family members was also documented.[22] A clinician recognized the initial outbreak in Marburg.[22] Thirty-one patients became infected, and seven died. The 23% human mortality and bizarre morphology of the newly discovered virus had a great psychological impact and led to new quarantine procedures for imported animals. During the next two decades, Marburg virus was associated with sporadic, isolated, usually fatal cases among residents and travelers in southeast Africa. In 1998–2000, an outbreak of Marburg HF in Durba, Democratic Republic of the Congo, was linked to individuals working in a gold mine.[23] In 2004–2005 there was a Marburg virus outbreak in Angola that caused over 200 deaths (90% mortality).[24]

Ebola viruses, taxonomically related to Marburg viruses, were first recognized during near-simultaneous explosive outbreaks in 1976 in small communities in the former Zaire (now the Democratic Republic of the Congo)[25] and Sudan.[26] Reuse of unsterilized needles and syringes and nosocomial contacts caused significant secondary transmission. These independent outbreaks involved serologically distinct viral species. The Ebola-Zaire outbreak involved 318 cases and 280 deaths (88% mortality), and the Ebola-Sudan outbreak involved 280 cases and 148 deaths (53% mortality). Since 1976 Ebola virus has appeared sporadically in Africa, causing several small- to mid-size outbreaks between 1976 and 1979. In 1995 a large epidemic of Ebola-Zaire HF involving 315 cases occurred, with an 81% case fatality rate, in Kikwit, a community in the former Zaire.[27] Meanwhile, between 1994 and 1996, the Ebola-Zaire virus caused smaller outbreaks in Gabon.[28] In 2000 Gulu, Uganda, suffered a large epidemic of VHF attributed to the Sudan species of Ebola virus.[29] More recently, Gabon and the Republic of Congo suffered small VHF outbreaks attributed to Ebola-Zaire virus. The most recent outbreaks in Gabon and the Republic of Congo also involved a catastrophic decline in populations of great apes, which may have a role in transmission to humans.[30,31]

In 1989 a third species of Ebola virus appeared in Reston, Virginia, in association with an outbreak of VHF among cynomolgus monkeys (*Macaca fascicularis*) imported to the United States from the Philippine Islands.[1] Hundreds of monkeys were infected (with high mortality) in this episode, but no human cases occurred, although four animal caretakers seroconverted without overt disease. Epizootics in cynomolgus monkeys recurred at other facilities in the United States and Europe through 1992 and again in 1996. The lack of human disease in these episodes suggests that the Reston species of Ebola may be less pathogenic to humans, although the pathogenic potential in humans is unknown. A fourth species of Ebola virus, Ivory Coast, was identified in Côte d'Ivoire in 1994; this species was associated with chimpanzees, and only one nonfatal human infection was identified.[32]

Little is known about the natural history of filoviruses. Surveys in Central Africa of a variety of species of animals and arthropods have yet to conclusively identify a reservoir host. Laboratory studies have shown that fruit and insectivorous bats can support

replication and circulation of high titers of Ebola virus without showing overt illness, suggesting that they could serve some role in the natural history of filoviruses.[33] Recently, an ecological study in Gabon and the Republic of the Congo showed asymptomatic infection by Ebola-Zaire virus in three species of fruit bats; however, no isolate was recovered from any of these bats.[34]

Flaviviridae

Viruses responsible for HF of the family *Flaviviridae* (type species yellow fever virus) are members of the genus *Flavivirus*, including yellow fever, dengue, Kyasanur forest disease, and Omsk. Mosquitoes transmit yellow fever, found throughout Africa and South America, and dengue, found throughout the Americas, Asia, and Africa.[35] Yellow fever was likely transported from Africa to the Americas during the slave trade. Yellow fever accounts in the Americas date to a probable 1648 outbreak in the Yucatan Peninsula. Carlos Finlay, a Cuban physician, identified *Aedes aegypti* as a likely vector and promulgated the theory of mosquito transmission. Dr Finlay supplied the Walter Reed commission with mosquito eggs and facilitated the US experiments that demonstrated that an extrinsic incubation period in the mosquito was needed before transmission. Benjamin Rush described classic dengue as "breakbone fever" in 1789. In 1954 dengue HF/dengue shock syndrome (DHF/DSS) was described in the Philippines, and became known as Philippine HF. There are four dengue virus serotypes: 1, 2, 3, and 4. DHF/DSS manifests in infants born to dengue-immune mothers, and in persons older than 1 year with prior immunity to one serotype of dengue virus who became infected with another serotype. Humans are the reservoir of dengue virus, but a jungle cycle involving forest mosquitoes and monkeys, similar to that associated with yellow fever, is recognized. In 1981 Cuba reported the first serologically confirmed case of DHF/DSS outside of Asia. Both yellow fever and dengue have had major impact on military campaigns and military medicine.

The tick-borne flaviviruses include the agents of Kyasanur forest disease of India[36] and Omsk HF found mainly in regions of Siberia.[37] Kyasanur forest disease, also called "monkey disease," was first described in 1957 in the Kyasanur forest of Mysore, India. Both diseases have a biphasic course; the initial phase includes a prominent pulmonary component, followed by a neurological phase with central nervous system manifestations. Both diseases can also manifest as HF. Alkhurma virus was isolated in 1995 from patients with HF in Saudi Arabia[38] and appears to be closely related to Kyasanur forest disease. Evidence suggests that transmission to humans can occur either by contamination of a skin wound with the blood of an infected vertebrate or bites of an infected tick, or by ingestion of unpasteurized contaminated milk.

Potential Role in Biological Warfare and Terrorism

Public concern about the dangers posed by VHFs reflects their potential for high morbidity and mortality, their potential spread from increased international commerce and air travel, and the heightened bioterrorism awareness advanced by the events surrounding September 11, 2001. The Centers for Disease Control and Prevention has classified most of the viruses causing HF as category A bioweapon agents.[39] This classification identifies agents associated with high mortality rates, ease of dissemination or person-to-person transmission, and potential for major public panic and social disruption, and that require special action for public health preparedness.

The Japanese studied VHF for use in warfare during their activities with Unit 731; specifically, they studied hantaviruses and noted that rodents served as reservoirs.[40] The Soviet Union, Russia, and the United States weaponized several HF viruses[41-43] and both the Soviet Union and Russia produced large quantities of Ebola, Marburg, Lassa, Junin, and Machupo viruses until 1992.[41,43] Soviet researchers determined that only a few virions of Marburg virus administered aerogenically can produce a lethal infection in monkeys,[44] and they showed that small doses of Ebola virus produced lethal infection in monkeys when administered by aerosol.[45] Many studies revealed that aerosol preparations of Ebola,[45,46] Marburg,[44,47] Lassa,[48] and Junin[49] viruses could produce lethal infection of nonhuman primates. Some argue that these viruses are too dangerous to develop as weapons because no effective vaccines or therapies exist; however, the Japanese cult Aum Shinrikyo's attempt in 1992 to obtain Ebola virus as part of a covert effort to develop biological weapons contradicts this view.[50]

Evidence suggests that North Korea weaponized yellow fever virus.[42,51] Moreover, the US offensive biological weapons program developed yellow fever and RVF viruses as weapons before terminating its program in 1969.[42] In 1970 the World Health Organization projected that an aerosol attack with 50 kg of RVF virus on a municipality of 500,000 residents would reach an estimated downwind distance of 1 km and cause 35,000 casualties, with a mortality rate of 0.5%.[52] Use of HF viruses with higher mortality rates such as Ebola virus or Marburg virus would ostensibly cause more significant morbidity and mortality.

The Working Group on Civilian Biodefense recently excluded the viruses causing dengue HF, CCHF, and the agents of HFRS as potential biological weapons.[53] The group excluded dengue virus because it is not transmissible by small-particle aerosol.[54] Exclusion of CCHF and the agents of HFRS as tools of bioterrorism is based primarily on technical problems; most importantly, these agents do not readily grow to high concentrations in cell culture, which is necessary for weaponization of an infectious organism.[53]

AGENT CHARACTERISTICS

Despite the diversity of the four families of viruses (*Arenaviridae*, *Bunyaviridae*, *Filoviridae*, and *Flaviviridae*) that contribute pathogens to the group of VHF agents, these viruses share common characteristics. The viruses causing severe HF have a single-stranded RNA genome and a lipid envelope, making them susceptible to detergents, as well as to low pH environments and household bleach. Conversely, they are stable at neutral pH, especially when protein is present. These viruses also are stable in blood for long periods and can be isolated from a patient's blood after weeks of storage at refrigerator or ambient temperatures. For example, Ebola virus was successfully cultured from dried blood found in syringes that had been stored at room temperature for about a month during a Central African outbreak in 1995.[55] Other examples include a study showing that yellow fever virus blotted onto filter paper discs, air dried, and stored at room temperature could be successfully cultured 90 days later.[56]

All HF viruses are biosafety level 3 or biosafety level 4 agents, except for the dengue viruses, because these viruses tend to be stable and highly infectious as fine-particle aerosols and produce disease with high morbidity and mortality. The HF viruses vary considerably in morphology from typical small isometric or moderately sized spherical virions to highly unusual pleomorphic or filamentous particles (Figure 13-1).

Arenaviridae

Arenavirus particles contain a genome consisting of two ambisense single-stranded RNA molecules, designated S (small) and L (large), of about 3.4 kb and 7.2 kb in length, respectively.[57] The S segment contains two genes that encode three structural proteins: the nucleoprotein (NP or N), and the envelope glycoproteins (GP1 and GP2). The L segment contains two genes that encode two proteins: the viral polymerase (L protein) and the Z protein. NP and L associate with the genomic RNA in a ribonucleoprotein complex or nucleocapsid structure. Z protein functions as a matrix protein and is responsible for the formation of viral particles.[58] GP1 and GP2 are initially synthesized as a precursor molecule, GPC, which is postranslationally cleaved.[59] GP2 homotetramers bind by ionic interactions with GP1 homotetramers, which make up the globular head of the glycoprotein spikes.[60] GP1 is the portion of the surface glycoprotein spike that is the effector for receptor binding,[61,62] whereas the GP2 is the viral fusion protein.[63,64]

Bunyaviridae

Bunyavirus particles contain three single-stranded RNA genome segments designated large (L), medium (M), and small (S), which vary in size among the genera.

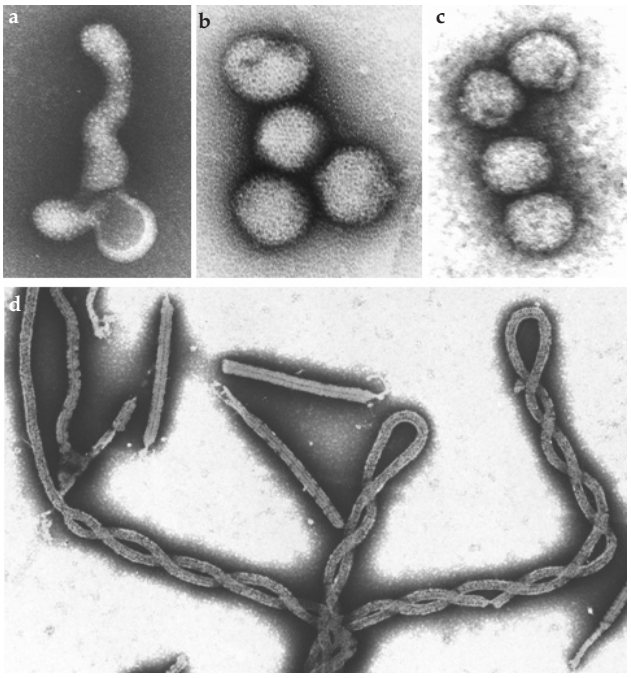

Fig. 13-1. Transmission electron micrographs of negatively stained hemorrhagic fever viral particles. (**a**) Junin virus. Arenavirus particles range in morphology from highly pleomorphic as shown in this field to mainly spherical. Virion sizes range from 50 to 300 nm with a mean of 100 to 130 nm. (**b**) Rift Valley fever virus. Bunyaviral particles are roughly spherical and range in diameter from 90 to 120 nm. (**c**) Yellow fever virus. Flaviviral particles are essentially isometric and consistent in size, ranging from 40 to 50 nm in diameter. (**d**) Ebola virus. Filoviral particles are mostly filamentous and vary in length up to 14,000 nm with a uniform diameter of 80 nm. Mean unit length is about 1,000 nm. Other forms of filoviral particles include U-shaped, "6"-shaped, or circular configurations; branching of filamentous particles can also occur.

The L segment encodes an RNA-dependent RNA polymerase (L), the M segment encodes two virion glycoproteins (G1 and G2) and in some viruses a nonstructural protein (NSm), and the S segment encodes a nucleoprotein (N) and in some viruses a nonstructural protein (NSs).[65-67] The structural proteins (L, N, G1, G2) are encoded in viral cRNA. NSs are encoded in the M segment cRNA and the S segment vRNA of phleboviruses. Hantaviruses and nairoviruses use negative-sense coding strategies, whereas phleboviruses use ambisense coding strategies. The functions of the NSs have not been fully delineated; NSs protein may control the activity of the viral polymerase and was proposed to block interferon (IFN) production.[68]

Filoviridae

Ebola and Marburg virus particles contain an approximately 19-kb, single, negative-stranded, linear RNA genome that is noninfectious. The genome encodes seven structural proteins with the following gene order: 3′ leader, nucleoprotein (NP), virion protein (VP) 35, VP40, glycoprotein (GP), VP30, VP24, polymerase L protein, and 5′ trailer.[69,70] Four of these proteins, NP, VP30, VP35, and L, associate with the genomic RNA in a ribonucleoprotein complex, whereas the three remaining proteins, GP, VP24, and VP40, are associated with the membrane. GP is the surface glycoprotein that forms the spikes on the virion and is the effector for receptor binding and membrane fusion.[71,72] GP is synthesized as a precursor molecule, GP_0, which is postranslationally cleaved by furin or a furin-like endoprotease into two subunits, GP_1 and GP_2; these subunits are linked by disulfide bonding to form a heterodimer.[73,74] Homotrimers of GP_1-GP_2 comprise the virion spikes. The unique organization of the GP gene of Ebola virus provides an important distinction between Marburg and Ebola viruses. The Marburg virus GP gene encodes a single product, the GP, in a conventional open reading frame, whereas all of the Ebola viruses encode the GP in two open reading frames that are expressed through transcriptional editing.[75,76] The primary gene product of the Ebola GP gene is not the GP, but rather a smaller, nonstructural, secreted glycoprotein (sGP), which is efficiently secreted from infected cells. VP40 functions as a matrix protein and is responsible for the formation of the filamentous particles.[77] VP24 is a minor viral protein whose functions remain unknown, but recent data indicate that VP24 possesses structural features consistent with viral matrix proteins and that it might have a role in viral assembly and budding.[78] VP24 and VP35 have been shown to play a role in interfering with type I IFN signaling (discussed below).

Flaviviridae

Flavivirus particles contain an approximately 11-kb, single, positive-stranded RNA genome. A single open reading frame is flanked by 5′ and 3′ noncoding regions and produces a large polyprotein that is cotranslationally and posttranslationally processed by cellular proteases into three structural proteins and seven nonstructural proteins in the order C-prM/M-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5.[79,80] The nucleocapsid is composed of a single capsid protein (C). In infected cells, the prM protein is cleaved by furin to form a small, nonglycosylated membrane protein (M) and an N-terminal "pr" segment that is secreted. E protein is a large glycosylated type I membrane protein. The remaining proteins are nonstructural proteins. The NS1 protein secreted from infected mammalian cells is thought to play a role in RNA replication.[81] The function of NS2A is unknown, but some data suggest that it may function in the recruitment of RNA templates to the membrane-bound replicase, or it could be involved in the inhibition of IFN.[82,83] NS2B is a small membrane-associated protein that forms a complex with NS3 and is a required cofactor of the serine protease function of NS3.[84] NS3, a large cytoplasmic protein that associates with membranes by interacting with NS2B, is thought to play a role in polyprotein processing and RNA replication.[84-88] NS4A and NS4B are membrane-associated proteins; NS4A appears to be involved in RNA replication,[82,88,89] and NS4B is also localized to sites of RNA replication and may be involved in inhibiting IFN signaling.[90] NS5 contains sequence homology similar to RNA-dependent RNA polymerases of other positive-stranded RNA viruses and also with methyltransferase enzymes involved in RNA cap formation.[91,92]

CLINICAL MANIFESTATIONS

Patients infected with these viruses may experience a wide spectrum of clinical manifestations with varying degrees of severity, yet not all patients develop classic VHF syndrome. The exact nature of the disease depends on the viral virulence and strain characteristics, routes of exposure, dose, and host factors. For example, DHF/DSS typically develops only in patients previously exposed to heterologous dengue serotypes.[93] As another example, for Ebola HF, the Zaire species is clearly more pathogenic in humans and nonhuman primates than the Sudan species, yet the incubation period reported for person-to-person transmission in

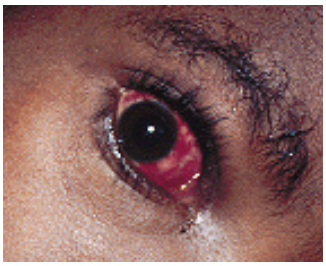

Fig 13-2. Ocular manifestations of viral hemorrhagic fever. Conjunctival injection and subconjunctival hemorrhage, as seen in this Lassa fever patient, are sometimes associated with viral hemorrhagic fever infection.
Photograph: Courtesy of Daniel G Bausch, MD, MPH&TM, Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana.

Ebola-Zaire infections greatly exceeds the incubation period for injections or needle stick accidents.[94]

A main target organ in VHF syndrome is the vascular bed; correspondingly, the dominant clinical features are usually a consequence of microvascular damage and changes in vascular permeability.[95] Common presenting complaints are fever, myalgia, and prostration; clinical examination may reveal only conjunctival injection, mild hypotension, flushing, and petechial hemorrhages. Fulminant VHF typically evolves to shock and generalized bleeding from the mucous membranes (Figure 13-2), and often is accompanied by evidence of neurological, hematopoietic, or pulmonary involvement. Hepatic involvement is common (Figure 13-3), but only a small percentage of patients with RVF, CCHF, Marburg HF, and Ebola HF manifest a clinical picture dominated by jaundice and other evidence of hepatic failure. Renal failure is proportional to cardiovascular compromise, except for patients with HFRS caused by hantaviruses, in which renal failure is an integral part of the disease process and oliguria is a prominent feature of the acutely ill patient.[96] VHF mortality may be substantial, ranging from 5% to 20% or higher in recognized cases. Ebola and Marburg outbreaks in sub-Saharan Africa have had particularly high case-fatality rates, ranging from 50% to 90%.[23-27]

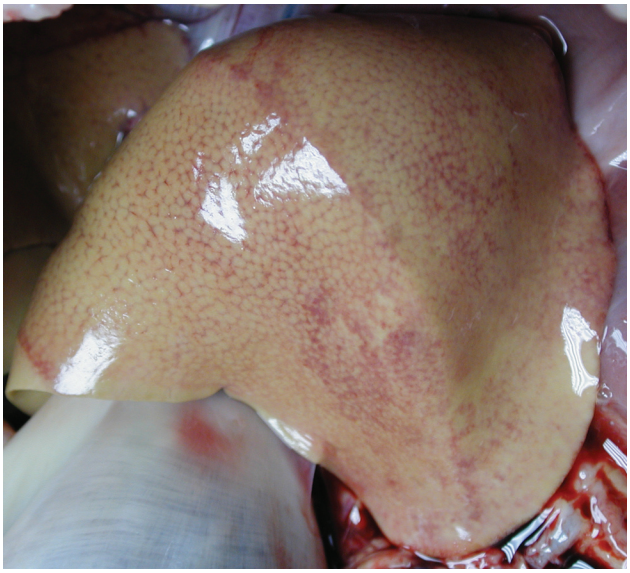

Fig 13-3. Liver pathology in viral hemorrhagic fever. Liver of a rhesus monkey experimentally infected with Marburg virus (Angola strain) showing diffuse reticulated pattern resulting from degeneration and necrosis.

The overall incubation period for VHF varies from 2 to 35 days. There is a prodrome period that may include a high fever, headache, malaise, myalgias, arthralgia, abdominal pain, nausea, and diarrhea, which usually lasts less than a week. The clinical characteristics vary with the viral agent involved. Filoviruses, flaviviruses, and RVF tend to have an abrupt onset, whereas arenaviruses have a more insidious onset. For Lassa fever patients, hemorrhagic manifestations are not pronounced, and neurological complications are infrequent, develop late, and manifest only in the most severely ill group. Deafness is a frequent consequence of severe Lassa fever. For the South American arenaviruses (Argentine and Bolivian HFs), neurological and hemorrhagic manifestations are much more prominent. RVF virus is primarily hepatotropic and hemorrhagic disease is infrequent. In recent outbreaks in Egypt, retinitis was frequently associated with RVF virus infection.[97]

Unlike RVF, in which hemorrhage is not prominent, infection with CCHF is usually associated with profound disseminated intravascular coagulation (DIC) (Figure 13-4). Patients with CCHF may bleed profusely, and because this occurs during the acute, viremic phase, contact with an infected patient's blood is a special concern. Several nosocomial outbreaks have been associated with CCHF virus.

The clinical picture for diseases caused by hantaviruses is evolving, especially now in the context of HPS. The pathogenesis of HFRS may be somewhat different; immunopathological events seem to be a major factor. When patients present with HFRS, they are typically oliguric. Surprisingly, the oliguria commences while the patient's viremia is resolving and patients are mounting a demonstrable antibody response. This occurrence has practical significance in that renal dialysis can be started with relative safety. Clinical data from human outbreaks caused by filoviruses are sparse. Although mortality is

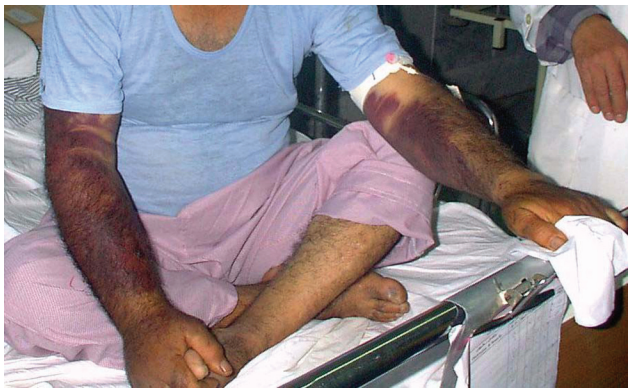

Fig. 13-4. Massive cutaneous ecchymosis associated with late-stage Crimean-Congo hemorrhagic fever viral infection, 7 to 10 days after clinical onset. Ecchymosis indicates multiple abnormalities in the coagulation system, coupled with loss of vascular integrity.
Photograph: Courtesy of Dr Sadegh Chinikar, Pasteur Institute of Iran, Tehran, Iran.

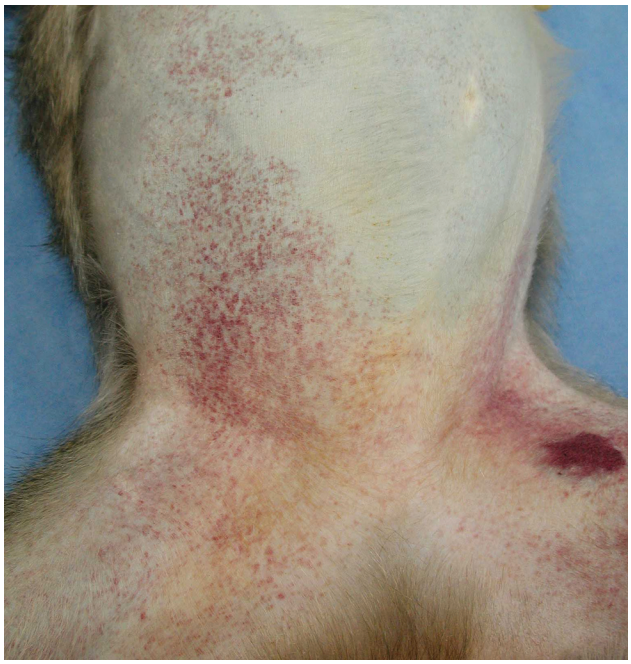

Fig. 13-5. Characteristic petechial rash of the abdomen and inguinal region of a cynomolgus monkey infected with Marburg virus. Note also the abnormalities in the coagulation system as evidenced by subcutaneous pooling of blood at a recent venipuncture site on animal's left inner thigh.

high, outbreaks are rare and sporadic. Marburg and Ebola viruses produce prominent maculopapular rashes in both human and nonhuman primates (Figure 13-5), and DIC appears to be a factor in their pathogenesis. Therefore, treating the DIC should be considered, if practicable, for these patients.

Among the flaviviruses, yellow fever virus is hepatotropic; black vomit caused by hematemesis has been associated with this disease. Patients with yellow fever develop clinical jaundice and die with something comparable to hepatorenal syndrome. Dengue HF and shock are uncommon, life-threatening complications of dengue, and are thought to result from an immunopathological mechanism triggered by sequential infections with different dengue viral serotypes (especially in children).[93] Although this is the general epidemiological pattern, dengue virus may also (rarely) cause HFs during primary infections and in adults.[98] Laboratory findings for VHFs may include thrombocytopenia (or abnormal platelet function) or leukopenia (except for Lassa fever, which includes leukocytosis). Some patients have anemia, and others have hemoconcentration; most have elevated liver-associated enzymes. Bilirubin is elevated in RVF and yellow fever. Prothrombin time, activated partial thromboplastin time, and bleeding time are often prolonged. Patients in DIC have elevated fibrin degradation products and decreased fibrinogen. Urine tests may show proteinuria and hematuria; patients with renal failure may have oliguria or azotemia. Blood, occult or overt, may be present in stools.

PATHOGENESIS

Understanding the kinetics of host–pathogen relationships and identifying critical pathogenetic processes are important for the rational development of prophylactic and therapeutic countermeasures. For the most part, the specific mechanisms underlying the pathogenesis of HF viral infection have not been clearly explained, although recent progress has been made, particularly on Ebola virus. A paradigm showing the current views on the pathogenesis of the HF viruses is illustrated in Figure 13-6. A central theme common to all VHFs, with the possible exception of yellow fever, is that lesions are not severe enough to account for terminal shock and death of the host. Yet VHF infections are characterized by a fulminant shock-like syndrome in fatal cases, suggesting that inflammatory mediators may play a determining role in the disease pathogenesis. Fatal HF viral infections are generally characterized by high viremia and immunosuppression. HF viral infection in humans and nonhuman primates is characterized by deleterious changes in lymphoid

tissues and defects in the coagulation system. Another common feature among these viruses is that all of the HF viruses appear to target and impair the cells that play the most critical roles in initiating the antiviral immune response, likely leading to unchecked and overwhelming viral burdens. To provide a better understanding of these pathogenic events, this section looks at the interactions between VHFs and the cells

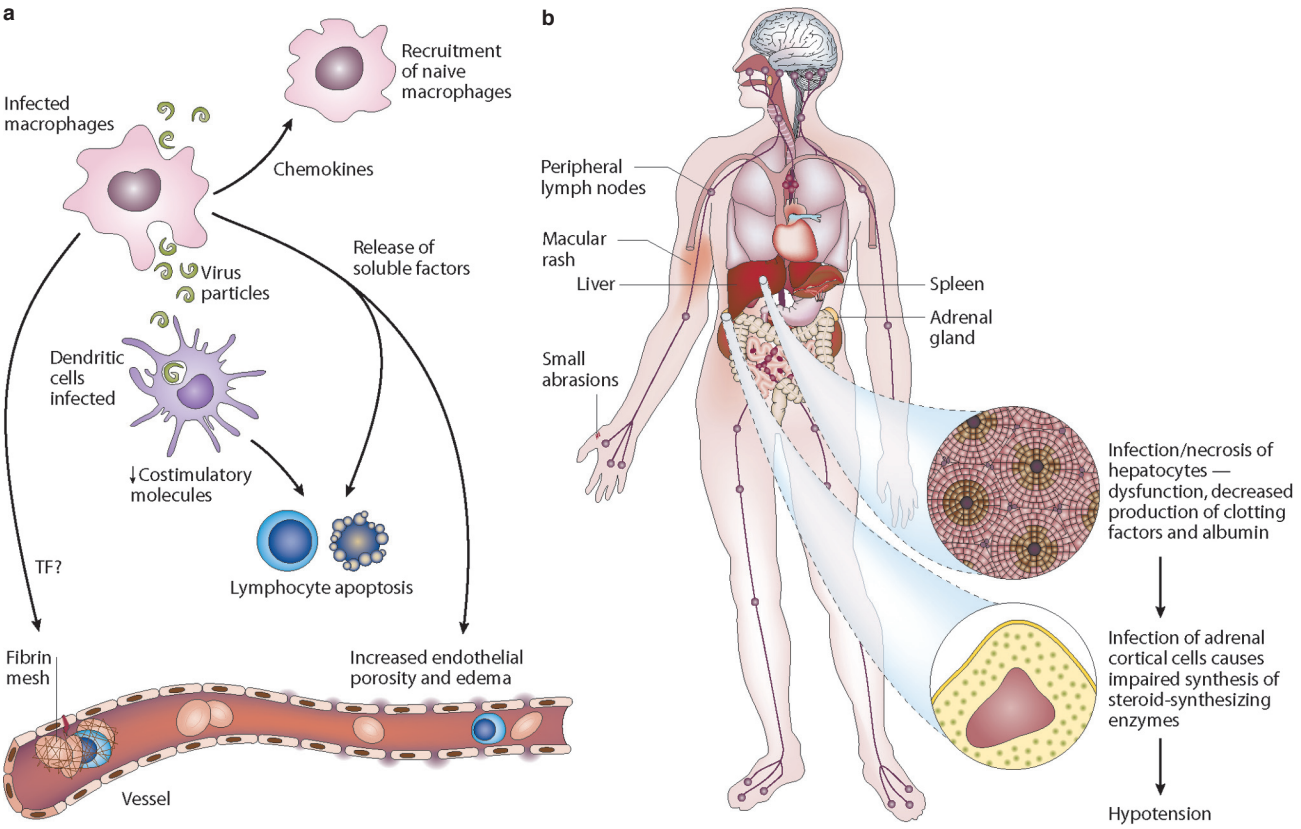

Fig. 13-6. Model of viral hemorrhagic fever (VHF) pathogenesis. (**a**) Virus spreads from the initial infection site to regional lymph nodes, liver, and spleen. At these sites, the virus infects tissue macrophages (including Kupffer cells) and dendritic cells. Soluble factors released from virus-infected monocytes and macrophages act locally and systemically. Release of chemokines from these virus-infected cells recruits additional macrophages to sites of infection, making more target cells available for viral exploitation and further amplifying the dysregulated host response. Although none of these viruses infects lymphocytes, the rapid loss of these cells by apoptosis is a prominent feature of disease. The direct interaction of lymphocytes with viral proteins cannot be discounted as having a role in their destruction, but the marked loss of lymphocytes is likely to result from a combination of factors, including viral infection of DC and release of soluble factors from virus-infected monocytes and macrophages. For example, viral infection of dendritic cells impairs their function by interfering with the upregulation of costimulatory molecules, which are important in providing rescue signals to T lymphocytes. Additionally, release of soluble factors from infected monocytes and macrophages results in deletion of lymphocytes, both directly by release of mediators such as nitric oxide, and indirectly by contributing to upregulation of proapoptotic proteins such as Fas and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). The coagulation abnormalities vary in nature and magnitude among the VHFs. For example, Ebola virus induces the overexpression of tissue factor (TF) that results in activation of the clotting pathway and the formation of fibrin in the vasculature. For example, coagulation disorders are less marked in Lassa fever, and impairment of endothelial function contributes to edema, which seems to be a more prominent finding in Lassa fever than in other VHFs. (**b**) The hemodynamic and coagulation disorders common among all of the VHFs are exacerbated by infection of hepatocytes and adrenal cortical cells. Infection of hepatocytes impairs synthesis of important clotting factors. At the same time, reduced synthesis of albumin by hepatocytes results in a reduced plasma osmotic pressure and contributes to edema. Impaired secretion of steroid-synthesizing enzymes by VHF-infected adrenal cortical cells leads to hypotension and sodium loss with hypovolemia. Macular rashes are often seen in VHFs. Reproduced with permission from Macmillan Publishers Ltd.: Geisbert TW, Jahrling PB. Exotic emerging viral diseases: progress and challenges. *Nat Med.* 2004;10(12 suppl):S110–121.

and tissues they affect as well as factors contributing to the immunological and hematological imbalances associated with HF viral infection. Dengue HF is not addressed in this section because of its complex nature (partly attributable to antibody-dependent enhancement), which does not appear to play a prominent role in other HF viral infections. Several researchers have reviewed the pathogenesis of dengue HF.[99-102]

Target Cells and Tissues

In general, the HF viruses all have a broad cell tropism, infecting a wide range of cell types. Immunohistochemistry and in situ hybridization analyses of tissues from fatal human cases or experimentally infected nonhuman primates show that monocytes, macrophages, dendritic cells (DCs), endothelial cells, hepatocytes, and adrenal cortical cells all generally support replication of these viruses.[103-116] The sequence of infection, however, is largely unknown. Systematic temporal studies in nonhuman primates experimentally infected with Ebola-Zaire virus suggest that monocytes, macrophages, and DCs are early and preferred targets of these viruses, whereas endothelial cells are infected much later during the course of disease, proximal to death.[114,115] Infection of endothelial cells appears to play a larger role in the pathogenesis of the hantaviruses than of the other HF viruses; although endothelial damage probably does not occur by direct effects of hantaviral replication.[117,118]

The mechanism (or mechanisms) of entry of the HF viruses into host cells has not been well characterized, but it is not believed to occur by direct fusion with the plasma membrane. Instead, researchers think that these viruses exploit the host cell's endocytic machinery to access the cytoplasm. Many different types of cell-surface binding proteins have been proposed to play a role in the entry of the viruses that cause VHF. For example, the asialoglycoprotein receptor of hepatocytes is postulated to serve as a binding protein for the Marburg virus,[119] whereas the folate receptor α and the DC-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN) and DC-SIGN-related factors have also been associated with the entry of the Marburg virus and the Ebola virus.[120-125] Moreover, the β1 integrin receptors and a human macrophage galactose- and *N*-acetylgalactosamine-specific C-type lectin are associated with the entry of Ebola virus.[124-125] β3 integrins might mediate entry of the several hantaviruses.[126] Alpha-dystroglycan was identified as an important receptor for Lassa virus,[127] but does not appear to be a receptor for the South American arenaviruses that cause HF.[128,129] Again, because these viruses have such a broad cell tropism, infecting a wide range of cell types, it is highly likely that they exploit many molecules for entry. Consistent with this notion, it has been proposed that Ebola virus uses a variety of different C-type lectins for efficient entry into host cells.[125]

The similar cell and tissue tropism among the VHFs suggests commonalities in the entry mechanisms. Findings in many laboratories have shown that the transmembrane proteins of many RNA viruses including Ebola and Lassa have common structural and functional elements essential for viral entry.[130] For example, these viruses share a coiled-coil type of entry protein. Researchers anticipate that these general principles may also apply to other VHFs.

The role of the endothelium in the pathogenesis of the VHFs has been a particularly controversial topic. Vascular damage can be induced by immunological mechanisms and/or by direct infection of the vascular tissue. Impairment of endothelial cell functions can cause a wide range of vascular effects that lead to changes in vascular permeability or hemorrhage. Several in-vitro and ex-vivo studies have suggested that the Ebola virus GP is cytotoxic and is a main determinant of vascular cell injury, thus implying that direct Ebola virus-replication-induced structural damage of endothelial cells triggers the hemorrhagic diathesis.[131,132] However, more recent in-vitro studies suggest that cell rounding and downregulation of surface markers are late events in Ebola infection, whereas synthesis and massive release of virions occur at early steps and do not cause significant cytotoxic effects.[133] These in-vitro findings are supported by in-vivo studies showing that Ebola infection of endothelial cells does not trigger cell death and that endothelial cell infection occurs only late in the disease course.[115] Likewise, in-vitro studies have shown that Lassa virus can replicate in human endothelial cells without damaging them.[134]

Scientists searched for the etiology of the hemorrhagic diatheses in fatal cases caused by Ebola virus and Marburg virus in tissues from the initial outbreaks in 1967 and 1976, respectively, but no vascular lesions were identified.[135,136] In a recent study, consistent with the original histology observations in fatal human cases, Geisbert and colleagues demonstrated that Ebola virus infection of endothelial cells does not extensively disrupt the architecture of the vascular endothelium in Ebola-infected cynomolgus monkeys.[115] As noted previously, although Ebola virus replicated in endothelial cells of these animals, endothelial cell infection was only seen focally at late stages of disease, after the onset of the hemorrhagic abnormalities that characterize Ebola HF. Although ultrastructural evidence of endothelial cell activation and disruption was observed at midpoint to end stages of disease, it was postulated that the vasoac-

tive effects on endothelial cells were mediated indirectly because these changes were not associated with the presence of intracytoplasmic Ebola viral antigens.[115] Feldmann and colleagues support the view that mediator release from filovirus-infected target cells can have deleterious effects on the endothelium.[137]

For other VHFs, endothelium may be affected in a manner similar to the paradigm presented for the filoviruses. No specific vascular lesions were found in 12 fatal cases of AHF,[138] nor were specific vascular lesions observed in rhesus monkeys experimentally infected with Machupo virus.[139] Endothelium was only minimally infected in rhesus monkeys experimentally infected with Lassa virus, and overt endothelial necrosis was not observed histologically.[140] As noted previously, the endothelium appears to play a more important role in hantavirus infections than in other VHFs; however, capillary leakage caused by hantavirus infection is thought to occur as a consequence of immune-mediated endothelial injury and not by direct effects of viral replication.[117,118]

In addition to the macrophage-rich lymphoid tissues, the liver and the adrenal gland appear to be important target organs for all HF viruses, and this tropism likely plays an equally important role in the disease pathogenesis. Various degrees of hepatocellular necrosis were reported in HF viral infections of humans and nonhuman primates[109,114,136,139,141-145]; however, as noted before, the hepatocellular lesions are generally not significant enough to explain the cause of death. The exception is yellow fever, in which the extent of direct liver injury in some cases is severe enough to account for the disease. Markers of hepatocellular injury and fulminant hepatic dysfunction such as circulating liver-associated enzymes (eg, aspartate aminotransferase, alanine aminotransaminase) directly correlate with severity of yellow fever infection and prognosis.[146] Elevations in liver-associated enzymes are prominent findings in most severe VHF infections.[6,22,70,105,114,116,147-155] Hemorrhagic tendencies could be related to decreased synthesis of coagulation and other plasma proteins resulting from severe hepatocellular necrosis. In addition, reduced synthesis of albumin may cause a reduction in plasma osmotic pressure and contribute to edema, which again appears to be a recurrent feature of severe cases of Lassa fever.[156,157]

Various degrees of adrenocortical infection and necrosis were reported in HF viral infections of humans and nonhuman primates.[105,111,114,144,158] The adrenal cortex plays an important role in controlling blood pressure homoeostasis. Impaired secretion of steroid-synthesizing enzymes leads to hypotension and sodium loss with hypovolemia, which are important elements that have been reported in nearly all cases of VHF.[70,116,141,149,159-161] This finding suggests that impairment of adrenocortical function by viral infection may play a particularly important role in the evolution of shock that typifies late stages of VHF.

Immunosuppression

For nearly all VHFs, various degrees of lymphoid depletion and necrosis are seen in spleen and lymph nodes of fatal cases and in experimentally infected nonhuman primates (Figure 13-7).[103,104,112,114,116,135,139,142,143,149,150,162-169] Although lymphoid tissues are principal targets for HF viral infection, there is usually little inflammatory cellular response in these tissues or other infected tissues. With the exception of the hantaviruses, lymphopenia appears to be the most consistent pathological finding among HF viral infections of humans and nonhuman primates.[109,114,152,162,167,169-179] Despite the significant loss of lymphocytes during HF viral infection, none of the HF viruses replicates in lymphocytes. For Ebola and Marburg viruses, large numbers of lymphocytes undergo apoptosis in humans and experimentally infected nonhuman primates,[114,180-182] partly explaining the progressive lymphopenia and lymphoid depletion at death. The prominence of tingible body macrophages in lymphoid tissues of rhesus monkeys experimentally infected with Junin virus suggests that apoptosis is also a primary factor in the loss of lymphocytes noted for other VHFs.[145]

The mechanism (or mechanisms) for the underlying apoptosis and loss of bystander lymphocytes during

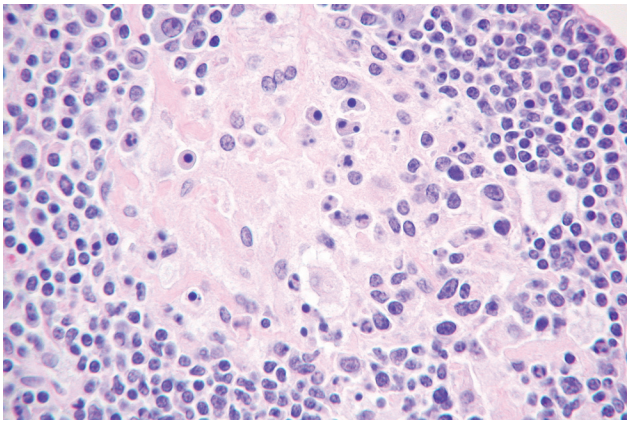

Fig. 13-7. Lymphoid depletion in viral hemorrhagic fever. Tonsil from a rhesus monkey experimentally infected with Ebola-Zaire virus, showing hyalinized follicle with typical depletion and apparent apoptosis of lymphocytes (hematoxylin-eosin stain, original magnification x 40).
Photograph: Courtesy of Dr Kelly Davis, Charles River Laboratories, Redfield, Arkansas.

the course of VHF illness has been unclear, but it is likely induced through multiple pathways. These pathways or processes may include the tumor necrosis factor-related, apoptosis-inducing ligand (TRAIL) and Fas death receptor pathways,[114,183] dysfunction of DCs induced by HF viral infection,[114,183-186] and abnormal production of proapoptotic soluble mediators such as nitric oxide (NO).[114,179,183,187]

Inflammatory Response

Cytokines, chemokines, and other inflammatory mediators function in a pleiotropic manner, acting on many different types of cells to modulate the host's immune response. Although cytokines and chemokines typically apply their antimicrobial actions locally, for example in areas of infection, cytokines and chemokines might also act systemically, and they commonly induce many of the symptoms of infection (eg, fever, myalgia). When present in high concentrations, cytokines and chemokines can have toxic or even lethal effects; studies of septic shock have associated abnormal production of pro-inflammatory cytokines and chemokines with disease severity and fatal outcome.[188-191]

HF viral infection of humans and nonhuman primates triggers the expression of many inflammatory mediators, including the IFNs; interleukin (IL)-6; IL-8; IL-10; IL-12; IFN-inducible protein-10; monocyte chemoattractant protein-1; regulated upon activation, normal T-cell expressed and secreted (RANTES); tumor necrosis factor-α, and reactive oxygen and nitrogen species.[6,114,179,180,183,187,192-205] Infection of many different primary human cells in vitro also shows that HF viral infection can trigger the production of many of these same inflammatory mediators.[115,137,183,206-210] Overall, it appears that virus-induced expression of these mediators results in an immunological imbalance that in part contributes to the disease progression. However, information regarding the inflammatory response after infection with the HF viruses is incomplete, and the existing data are often inconsistent. For example, high levels of IFN-α were reported in acute phase sera of patients infected with Ebola virus in one study[199] but not detected in a subsequent yet similar study.[187] Such contradictions also confound interpretation of some in-vitro data. The differences in profiles of circulating cytokines and chemokines among the VHFs may be attributable to factors other than the differences among the viruses, such as genetic differences among patients and, in particular, differences related to the disease phase when the samples were obtained.

Researchers have postulated that for patients with asymptomatic, nonfatal Ebola virus infection, the infection is controlled by an initial increase in cytokines, including IL-1β, IL-6, and tumor necrosis factor-α, which is followed by a return to preexposure levels.[211] Protection against fatal outcome for other VHFs may likewise depend on an early and robust cytokine response, but this remains to be established. Conversely, disease severity may also be increased by an inappropriate proinflammatory response early in the disease course. Thus, the delicate balance between protective and deleterious cytokine and chemokine responses remains to be defined for all the VHF agents.

In general, the type I IFN response appears to play an important role in the pathogenesis of the HF viruses, especially for RVF. A delayed IFN response was correlated with increased mortality in a rhesus monkey model of RVF.[152] In AHF patients, circulating levels of IFN-α are unusually high and during the acute phase are significantly higher in fatal cases than in survivors.[193] As noted previously, inconsistencies were reported in circulating levels of IFN-α in Ebola-infected patients; however, very high plasma levels of IFN-α were observed in experimentally infected monkeys.[114,183] The role of IFNs in Lassa fever is unclear. Several studies have evaluated resistance of Lassa virus to IFNs in vitro. In one study, Lassa was shown to be resistant to IFN-α,[212] and in a more recent study, IFN-α and IFN-γ were shown to inhibit the replication of Lassa virus.[213] Less is known about the role of IFNs in other VHFs, although a recent study suggested that IFN-α inhibits CCHF in vitro.[214] A significant concern when interpreting and comparing results from any of these VHF studies is the observation that there are 12 different subtypes of IFN-α, and previous studies have shown that the antiviral activities of the subtypes vary greatly.[215] The research on HF viruses has evaluated only total levels of IFN-α, and no researchers have dissected out which subtypes are represented in the plasma of infected patients and which subtypes may or may not have antiviral properties.

The ability of these viruses to directly modulate the host inflammatory response has yet to be fully delineated. Again, more research has been done on Ebola virus than any other VHF. The Ebola viral protein VP35 reportedly functions as a type I IFN antagonist.[216-218] Recent studies show that VP35 prevents IFN regulatory factor activation by inhibiting phosphorylation, and it is likely that VP35 prevents transcription of IFN-β.[217] Other studies suggest that Ebola viral protein VP24 expression might also interfere with type I IFN signaling.[218,219] RVF virus also has a viral protein, NSs, that functions as a type 1 IFN antagonist.[220] Based on studies with dengue virus, researchers think that flaviviruses including yellow fever virus inhibit type I IFN signaling primarily by the NS4B protein.[90,221] Little is known about whether other VHFs possess analogous

proteins that interfere with type I IFNs, but this possibility merits further study.

Although not extensively reported, one consistency in the proinflammatory response among the VHFs is the potential importance of reactive oxygen and nitrogen species in the disease pathogenesis. Increased blood levels of NO were reported in nonhuman primates experimentally infected with Ebola virus[114,183] and recently confirmed in Ebola-infected patients.[179,187] Sanchez and colleagues associated increased blood levels of NO with mortality.[179] Significant blood levels of NO have also been demonstrated in AHF patient sera[209] and in patients infected with hantaviruses.[195,202] Abnormal NO production has been associated with many pathological conditions, including apoptosis of bystander lymphocytes (as noted previously), tissue damage, and loss of vascular integrity, which may contribute to virus-induced shock. NO is known to have both protective and caustic effects, and this autotoxic overproduction may represent the host's endogenous counter-regulatory mechanism of protection against noxious agents, in this case the VHF viruses. In general, microbes induce monocytes and macrophages to produce NO in an attempt to control infection. However, in the case of the HF viruses, monocytes and macrophages are preferred target cells for viral replication. Enhanced replication in these cells may in turn exacerbate disease by producing large amounts of NO, resulting in deleterious effects, such as suppressive effects on lymphocyte proliferation and damage to other cells. NO is an important mediator of hypotension,[222,223] and, as noted previously, hypotension is a prominent finding among most of the VHFs.[70,116,141,149,159-161] Together, the information collected suggests that an impaired and ineffective immune response leads to high levels of virus and proinflammatory mediators in the late stages of disease, which is important in the pathogenesis of hemorrhage and shock in VHFs.

Coagulation Abnormalities

Abnormalities in blood coagulation and fibrinolysis during VHF infection are manifested as petechiae, ecchymoses, mucosal hemorrhages, and uncontrolled bleeding at venipuncture sites. However, massive loss of blood is atypical and, when present, is largely restricted to the gastrointestinal tract. Even in these cases, blood volume loss is insufficient to account for death. DIC is a syndrome characterized by systemic intravascular activation of coagulation leading to widespread deposition of fibrin in the vasculature, which contributes to the development of multiple organ failure.[224-226] DIC is associated with both bleeding and thrombotic abnormalities, and widespread thrombosis and bleeding commonly occur simultaneously. The occurrence of DIC in HF viral infection is the subject of much debate, and information that supports or refutes the presence of DIC is inconclusive. In general, DIC appears to be more prominent in Ebola HF and CCHF than among the other VHFs. The presence of DIC in any of the VHFs appears to strongly correlate with a poor outcome. For the purposes of this review, the authors can clearly state that regardless of whether DIC is an important and consistent feature among all VHFs, impairment of the coagulation system ostensibly contributes to the disease pathogenesis of all of these VHFs. Both coagulation and fibrinolysis appear to be activated by HF viral infection, and the degree of impairment of the coagulation system seems to be associated with the balance between these counteracting processes by the host.

Most VHF infections in humans and in nonhuman primate models are characterized by cutaneous flushing or macular rashes; however, the characteristics of these rashes vary among the VHFs. For example, nonpruritic petechial skin rashes on the axillae and groins, forehead, and chest appear in up to 50% of patients infected with Ebola or Marburg viruses and are more evident in patients with light-colored skin.[22,25-27,227,228] This same type of rash evolves in more than 50% of nonhuman primates (of the genus *Macaca*) experimentally infected with Ebola or Marburg viruses.[109,114,153,171,173,229,230] Petechial skin rashes are also associated with yellow fever.[116] In general, arenavirus infections in humans and in nonhuman primate models are typically characterized by flushed, erythematous rashes on the face and thorax[139,145,231,232]; although oral and axillary petechia are frequently observed in human cases of AHF.[233] For many VHFs, petechiae are sometimes observed on visceral organs.[114,116,140] In addition, congestion of various organs is a frequent finding at autopsy or necropsy.[109,114,141,143,156,165]

Thrombocytopenia appears to be a consistent finding among VHF infections of humans and nonhuman primates,[10,22,108,116,135,138,141,151,152,155,160,168,170,172,173,179,230,234-239] with the notable exception of Lassa fever.[105,140,162,240,241] Moderate thrombocytopenia was reported in patients with severe Lassa fever, but the most significant changes were noted in platelet function, which was markedly depressed in these patients.[149] Marked changes in platelet function have also been observed in Lassa-infected rhesus macaques[162] and in rhesus monkeys experimentally infected with yellow fever.[116] Researchers have postulated that the thrombocytopenia seen in the South American VHFs results in part from maturation arrest of megakaryocytes attributable to the high levels of IFN in these patients.[193] Similar inferences have been made for yellow fever.[116]

Histological and biochemical evidence of impairment of the coagulation system has been shown for many VHFs, but the data are largely incomplete and paradoxical. More is known about Ebola and Marburg viruses than the other VHFs. Fibrin deposition has been documented at autopsy for Marburg HF,[136,242] and clinical laboratory data suggest that DIC is an important feature of human Ebola and Marburg HF.[25,243] Numerous studies have shown histological and biochemical evidence of DIC during Ebola infection in a variety of nonhuman primate species, including significant changes in markers of blood coagulation and fibrinolysis, such as various clotting factors, fibrin degradation products (FDPs), D-dimers, protein C, tissue plasminogen activator, and urokinase plasminogen activator.[109,114,153,171,230,239] Fibrin and fibrinocellular thrombi in vessels in numerous tissues and fibrin deposits in the red pulp and marginal zone of the spleen are frequent findings in Ebola virus-infected cynomolgus and rhesus macaques.[107,109,230,239,244]

AHF infections are characterized by significant changes in markers of blood coagulation and fibrinolysis, including thrombin-antithrombin complexes, prothrombin fragments, protein C, D-dimers, tissue plasminogen activator, and plasminogen activator inhibitor-1.[237] Increased fibrinogen levels were detected in a study of 32 AHF patients, but FDPs were not detected, and DIC did not appear to be a relevant factor in these cases.[233] Three of 12 AHF cases showed intravascular fibrin thrombi and clinical features consistent with DIC.[138] Neotropical primates experimentally infected with Junin virus showed an increase in the prothrombin time and increases in circulating levels of fibrinogen and FDPs.[236] Prolongation of the activated partial thromboplastin time was noted in rhesus monkeys experimentally infected with Machupo virus, but evidence for DIC was inconclusive because of equivocal changes in fibrinogen levels and levels of FDPs.[235] Microscopic evidence of DIC was noted in only 1 of 10 rhesus monkeys experimentally infected with Machupo virus.[139]

Evidence of DIC has been reported in fatal cases of CCHF. Values for prothrombin ratio, prothrombin time, activated partial thromboplastin time, and FDPs were grossly elevated in 15 fatal cases, but fibrinogen and hemoglobin levels were depressed.[151] Many of these clinical pathologic changes were evident at an early stage of disease and had a highly predictive value for fatal outcome in the 35 monitored cases. Experimental infection of rhesus monkeys with RVF virus does not produce a uniformly lethal disease. However, as in human cases, the degree of hemorrhagic manifestations is associated with fatal outcome. Not surprisingly, changes in circulating levels of clotting factors, activated partial thromboplastin time, prothrombin time, FDPs, and evidence of DIC were more prominent in 3 of 17 RVF virus-infected monkeys that succumbed to challenge than animals that survived.[150,152] Multiple fibrin thrombi were present within the glomeruli and small intertubular vessels of these experimentally infected rhesus monkeys. In addition, fibrillar material that stained positive for fibrinogen was abundant in the spleen. Biochemical evidence of DIC has been noted in about half of the cases of Korean HF,[245] and microscopic evidence of alveolar fibrin was reported in cases of HPS.[246] DIC also appears to play a role in yellow fever; changes in clotting and prothrombin times, clotting factors, fibrinogen levels, and FDPs have been reported.[116,247]

DIC does not appear to be involved in Lassa fever. Microscopic hemorrhagic diathesis is rare, and the absence of fibrin thrombi correlates with generally normal measurements of coagulation mechanisms.[140,240] In one report, however, splenic necrosis that centered in the marginal zone was accompanied by the deposition of fibrin.[140] In rhesus monkeys experimentally infected with Lassa virus, several groups reported no changes in circulating levels of clotting factors and no evidence of DIC.[105,162,241]

The mechanism (or mechanisms) for triggering the coagulation abnormalities seen in VHF has not been fully delineated. Some of the latest studies on Ebola virus have begun to shed light on the pathogenesis of coagulation system dysregulation and suggest that development of coagulation abnormalities might occur much earlier than previously thought. Although it is likely that the coagulopathy seen in Ebola HF is caused by multiple factors, particularly during the later stages of disease, recent data strongly implicate tissue factor expression/release from Ebola-infected monocytes and macrophages as a key factor that induces the development of coagulation irregularities.[239] Levels of expression of tissue factor may also be affected by the production of proinflammatory cytokines, which (as noted previously) are induced in most HF viral infections. For example, IL-6 has been shown to effectively upregulate expression of tissue factor on monocyte[248,249] and endothelial cells.[250] Ruf recently reviewed the role of tissue factor in VHFs.[251] Other factors speculated to contribute to the coagulopathy seen in Ebola HF include impairment of the fibrinolytic system as evidenced by rapid declines in plasma levels of protein C during the course of infection in cynomolgus monkeys.[239] Reduced plasma levels of protein C were also seen in AHF patients.[237] Future studies are needed to further define and clarify the role of tissue factor and the protein C system in VHF.

Future Directions in Pathogenesis

Several recent developments in biomolecular technology will play major roles in future studies designed to elucidate the molecular mechanisms of these devastating diseases. One key breakthrough has been the successful development of reverse genetics systems for the generation of many HF viruses including infectious Ebola virus,[252,253] Marburg virus,[254] CCHF virus,[255] RVF virus,[256] and yellow fever virus.[257] These infectious clone systems will have a tremendous impact on the ability to identify key regulatory elements and structure–function relationships in the HF viral life cycles. Another technology that will facilitate the ability to dissect the pathogenesis of HF viral infection is cDNA microarrays. A genomic view of systemic interactions that occur during HF viral infection will provide clues to important host–virus interactions. A recent application of cDNA microarrays to experimental Ebola virus infections in nonhuman primates revealed prominent induction of NFk-β and tumor necrosis factor-α–regulated genes for Ebola, in contrast to negligible expression during variola virus infection.[258] Similarly, another recent microarray analysis demonstrated that Ebola and Marburg virus infection of human liver cells in vitro resulted in changes in expression of many genes associated with the immune system, coagulation, and acute-phase proteins.[259] Comparative analysis among the VHFs, which may reveal differentially expressed genes unique to each agent, could have diagnostic utility by identifying markers of disease progression and predictors of outcome, as well as improving the understanding of disease pathogenesis.

DIAGNOSIS

Differential Diagnosis

In the event of a covert bioterrorist attack, a high degree of suspicion would be required for any realistic chance of rapid VHF diagnosis. Whether clinicians would initially recognize VHF is unclear, but a cluster of such cases would likely alert clinicians to this possibility. Under natural conditions, these viruses have a geographically restricted distribution linked to the ecology of the reservoir species and vectors; thus, a detailed travel history is critical in making the VHF diagnosis. Patients with arenaviral or hantaviral infections often recall seeing rodents during the presumed incubation period; however, as the viruses spread to humans by aerosolized excreta or environmental contamination, actual contact with the reservoir is not necessary. Large mosquito populations are common during the seasons when RVF virus and the flaviviruses are transmitted, but a history of mosquito bite is sufficiently common to be of little assistance in making a diagnosis, whereas tick bites or nosocomial exposures are of some significance when CCHF is suspected. History of exposure to animals in slaughterhouses should raise suspicions of RVF and CCHF in a patient with VHF.

The variable clinical presentation of these diseases presents a major diagnostic challenge. VHF should be suspected in any patient presenting with a severe febrile illness and evidence of vascular involvement (subnormal blood pressure, postural hypotension, petechiae, hemorrhagic diathesis, flushing of the face and chest, nondependent edema), who has traveled to an endemic area or to someplace where intelligence suggests a biological warfare or terror threat. Signs and symptoms suggesting additional organ system involvement are common (headache, photophobia, pharyngitis, cough, nausea or vomiting, diarrhea, constipation, abdominal pain, hyperesthesia, dizziness, confusion, and tremor), but they rarely dominate the picture. The macular eruption characteristic of Marburg and Ebola HFs has considerable clinical importance.

As previously noted, laboratory findings can be helpful, although they vary from disease to disease, and summarization is difficult. Leukopenia may be suggestive, but in some patients, white blood cell counts may be normal or even elevated. Thrombocytopenia is a component of most VHF diseases, but to a varying extent. In some patients, platelet counts may be near normal, and platelet function tests are required to explain the bleeding diathesis. A positive tourniquet test has been particularly useful in diagnosing dengue HF, but this sign may be associated with other VHFs as well. Proteinuria or hematuria or both are common in VHF, and their absence virtually rules out AHF, Bolivian HF, and HFRS. Hematocrits are usually normal, and if there is sufficient loss of vascular integrity, perhaps mixed with dehydration, hematocrits may be increased. Soluble cytosolic liver-associated enzymes such as aspartate aminotransferase are frequently elevated. HF viruses are not primarily hepatotropic, but the liver is involved, and an elevated aspartate aminotransferase may help to distinguish VHF from a simple febrile disease.

For much of the world, the major differential diagnosis is malaria. Parasitemia in patients partially immune to malaria does not prove that malaria is responsible for symptoms. Typhoid fever, rickettsial,

and leptospiral diseases are major confounding infections; nontyphoidal salmonellosis, shigellosis, relapsing fever, fulminant hepatitis, and meningococcemia are some of the other important diagnoses to exclude. Establishing the cause of DIC is difficult and often confusing. Many conditions that cause DIC, such as acute leukemia, lupus erythematosus, idiopathic or thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome, could be mistaken for VHF.

Specific Diagnosis

Definitive diagnosis in an individual case rests on specific virological diagnosis. Most patients have readily detectable viremia at presentation (the exception is those with hantaviral infections). Infectious virus and viral antigens can be detected and identified by many assays of fresh or frozen serum or plasma samples or whole blood. Likewise, early immunoglobulin M antibody responses to the VHF-causing agents can be detected by enzyme-linked immunosorbent assays (ELISAs), often during the acute illness. Diagnosis by viral cultivation and identification requires 3 to 10 days for most VHFs (longer for the hantaviruses); with the exception of dengue, specialized microbiologic containment is required for safely handling these viruses.[260] Appropriate precautions should be observed in collecting, handling, shipping, and processing diagnostic samples (see "Packaging Protocols for Biological Agents/Diseases" at http://www.bt.cdc.gov/agent/vhf/index.asp). Both the Centers for Disease Control and Prevention and the US Army Medical Research Institute of Infectious Diseases (USAMRIID, Fort Detrick, Maryland) have diagnostic laboratories operating at the maximum biosafety level 4. Virus isolation should not be attempted without biosafety level 4 containment.

In contrast, most antigen-capture and antibody-detection ELISAs for these agents can be performed with samples that have been inactivated by treatment with beta-propiolactone[261] or gamma rays. Diagnostic tests based on reverse transcriptase polymerase chain reaction (RT-PCR) technology are safely performed on samples after RNA extraction using guanidium-based solutions. RT-PCR has been successfully applied to the real-time diagnosis of most of the VHF agents and is now the most widely used assay for identifying suspected VHF.[262-266] Recently, a multiplex PCR assay in which microbial gene products are coded by a library of 64 distinct mass tags was developed and shown to be capable of differentiating 10 different agents of VHF.[267] RT-PCR is particularly useful in cases where isolation of the infectious virus is difficult or impractical. RT-PCR has proven to be extremely valuable, for example, with HPS, in which Sin Nombre virus was recognized by PCR months before it was finally isolated in culture.[20] In cases where the identity of an agent causing suspected VHF is completely unknown, isolation in cell culture and direct visualization by electron microscopy, followed by identification by immunohistochemical procedures is frequently successful.[1,268] Filoviruses and arenavi-

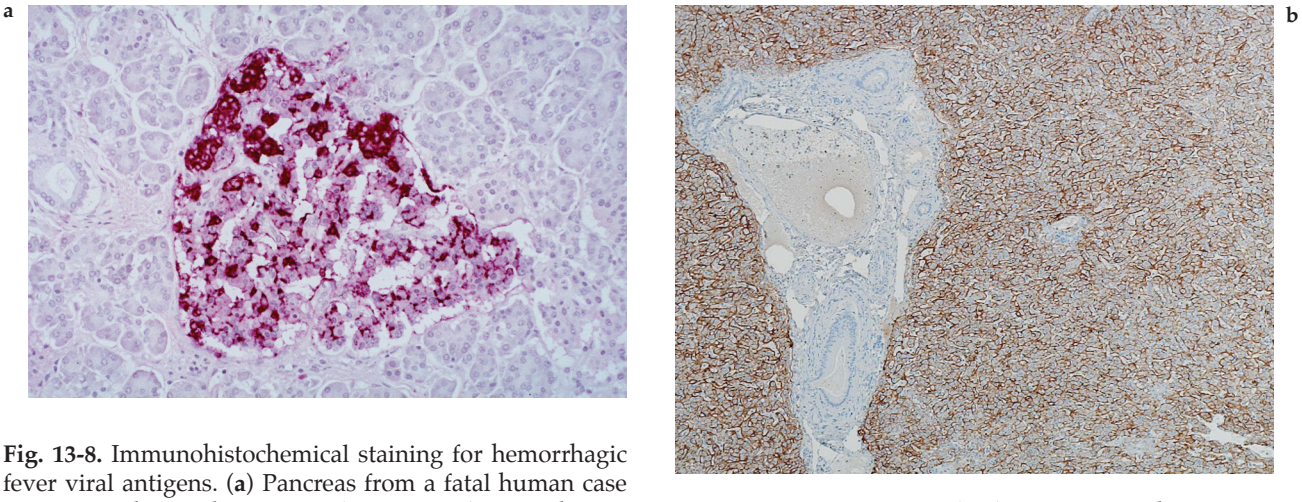

Fig. 13-8. Immunohistochemical staining for hemorrhagic fever viral antigens. (**a**) Pancreas from a fatal human case of Marburg hemorrhagic fever (Ravn strain). Note that Marburg virus–positive staining (red) is limited to the pancreatic islet, with multifocal distribution within the islet. (Streptavidin-alkaline phosphatase method, section counterstained with hematoxylin; original magnification x 20.) (**b**) Liver of a rhesus monkey experimentally infected with Marburg virus (Angola strain). Note intense and widespread Marburg virus–positive staining (brown) of hepatocytes with little immunostaining in portal area. (Immunoperoxidase method, original magnification x 10.)
Photograph b: Courtesy of LTC Tom Larsen, Pathology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland.

ruses induce intracytoplasmic viral inclusions that are morphologically unique to each viral family. Moreover, Ebola and Marburg viruses can be distinguished from each other by their distinctive viral inclusion material[269]; trained microscopists can distinguish these filoviral genera by the size and shape of the viral particles.[269] Immunohistochemical stains can be used to detect HF viruses in tissue sections. The application of immunohistochemical stains to skin specimens can provide a comparatively rapid diagnosis.[270] Immunohistochemical techniques are also useful for retrospective diagnosis of formalin-fixed tissues, where viral antigens can be detected and identified using batteries of specific immune sera and monoclonal antibodies (Figure 13-8).

MEDICAL MANAGEMENT

Patients with VHF syndrome require close supervision, and some require intensive care. Because the pathogenesis of VHF is not entirely understood and availability of antiviral drugs is limited, treatment is largely supportive. This care is essentially the same as the conventional care given to patients with other causes of multisystem failure. The challenge with VHF patients is to provide this support while minimizing the risk of infection to other patients and medical personnel.

Supportive Care

Patients with VHF syndrome generally benefit from rapid, atraumatic hospitalization to prevent unnecessary damage to the fragile capillary bed. Transporting these patients, especially by air, is usually contraindicated because of the effects of drastic changes in ambient pressure on lung water balance. Frequently patients manifest restlessness, confusion, myalgia, and hyperesthesia; these conditions should be managed by reassurance and other supportive measures, including the judicious use of sedatives, pain relief, and amnestic medications.

Secondary infections are common and should be sought and aggressively treated. Concomitant malaria should be treated aggressively with a treatment regimen known to be effective against the geographical strain of the parasite; however, the presence of malaria, particularly in immune individuals, should not preclude management of the patient for VHF syndrome if such treatment is clinically indicated.

Intravenous lines, catheters, and other invasive techniques should be avoided unless they are clearly indicated for appropriate management of the patient. Attention should be given to pulmonary toilet, the usual measures to prevent superinfection, and the provision of supplemental oxygen. Treatment with steroids or other agents that cause generalized immunosuppression has no empirical basis and is contraindicated, except possibly in treatment of HFRS.

The diffuse nature of the vascular pathological process may lead to a requirement for support of several organ systems. Myocardial lesions detected at autopsy reflect cardiac insufficiency antemortem. Pulmonary insufficiency may develop, and, particularly with yellow fever, hepatorenal syndrome is prominent.[35]

Treatment of Bleeding

The management of bleeding in VHF cases is controversial. Uncontrolled clinical observations support vigorous administration of fresh frozen plasma, clotting factor concentrates, and platelets, as well as early use of heparin for prophylaxis of DIC. In the absence of definitive evidence of VHF disease or DIC, mild bleeding manifestations should not be treated. More severe hemorrhage requires appropriate replacement therapy. When there is definitive laboratory confirmation of DIC, heparin therapy may be considered if appropriate laboratory support is available. Supportive strategies directed toward inhibiting coagulation activation may be warranted and have been shown to be beneficial in experimental and initial clinical studies.[271] Many new modalities to manage the pronounced coagulopathy that typifies many VHFs are being evaluated, most notably in nonhuman primate models of Ebola HF (discussed below).

Treatment of Hypotension and Shock

Management of hypotension and shock is difficult. Patients often are modestly dehydrated from heat, fever, anorexia, vomiting, and diarrhea, in any combination. There is extensive loss of intravascular volume through hemorrhage and increased vascular permeability.[272] Nevertheless, these patients often respond poorly to fluid infusions and readily develop pulmonary edema, possibly from myocardial impairment and increased pulmonary vascular permeability. Asanguineous fluids (either colloid or crystalloid solutions) should be given, with caution. Although it has never been evaluated critically for VHFs, dopamine might be the agent of choice for patients with shock who are unresponsive to fluid replacement. Alpha-adrenergic vasoconstricting agents

have not been clinically helpful except when emergent intervention to treat profound hypotension is necessary. Vasodilators have not been systematically evaluated. Pharmacological doses of corticosteroids (eg, methylprednisolone 30 mg/kg) provide another possible, but untested, therapeutic modality in treating shock.

Isolation and Containment

Patients with VHF syndrome (with the exception of dengue and classical hantavirus disease) generally have significant quantities of virus in their blood, and perhaps in other secretions as well. Secondary infections among contacts and medical personnel not parenterally exposed are well documented. Thus, caution is needed when evaluating and treating patients with suspected VHF syndrome. Overreaction by medical personnel is inappropriate and detrimental to both the patient and staff, but it is prudent to provide isolation measures as rigorous as feasible.[273] At a minimum, isolation measures should include the following:

- Restricted access to the patient and use of stringent barrier nursing including mask, gown, glove, and needle precautions.
- Proper hazard labeling of all specimens submitted to the clinical laboratory with notification of appropriate clinical personnel.
- Proper disposal of all material within the isolation room by autoclaving or liberal disinfection of contaminated materials using such disinfectants as hypochlorite or phenols.

For more intensive care, however, increased precautions are recommended. Members of the patient-care team should be limited to a small number of selected, trained individuals, and special care should be directed toward eliminating all parenteral exposures. Use of endoscopy, respirators, arterial catheters, routine blood sampling, and extensive laboratory analysis increases opportunities for aerosol dissemination of infectious blood and body fluids. For medical personnel, wearing flexible plastic hoods equipped with battery-powered blowers provides excellent protection of the mucous membranes and airways.

PREVENTION AND CONTROL

Active Vaccination

With the possible exception of yellow fever, outbreaks of VHF have been relatively infrequent, small in size compared to other infectious diseases, and confined largely to remote geographic locales; quarantine of sick patients has been effective in controlling epidemics. In the past, this small global market has generated little commercial interest for developing VHF vaccines. However, the increased concern about the potential of these viruses as biological weapons and the recent attention drawn to outbreaks of emerging and reemerging viruses, such as the 2004–2005 epidemic of Marburg HF in Angola, has dramatically changed perspectives on the need for VHF vaccines.

The only established and licensed virus-specific vaccine available against any of the HF viruses is the yellow fever live attenuated 17D vaccine, which is mandatory for travelers to endemic areas of Africa and South America (Table 13-2).[274] For prophylaxis against AHF virus, a live attenuated Junin vaccine strain (Candid #1) was developed at USAMRIID[275] as part of an international cooperative project (USAMRIID-Pan American Health Organization) and is available as an investigational new drug (IND). Candid #1 was proven to be effective in phase III studies in Argentina,[276] and plans are proceeding to obtain a new drug license. Candid #1 elicits high levels of protective antibodies lasting 9 years in approximately 90% of the people vaccinated with a single dose. This vaccine also protects against Bolivian HF in experimentally infected primates. Two IND vaccines were developed against RVF: a formalin-inactivated vaccine that requires three boosters, which has been in use for 20 years, and a live attenuated RVF viral strain (MP-12). The inactivated vaccine has been administered to laboratory workers and appears to be safe and efficacious, but the ability to produce this vaccine in the United States no longer exists.[277]

For the hantaviruses, five commercially available vaccines are being produced in China,[278] but these vaccines are not generally considered acceptable by United States standards. Another USAMRIID product, a genetically engineered vaccinia construct expressing hantaviral structural proteins, is in phase II safety testing in US volunteers. A formalin-inactivated Kyasanur forest disease vaccine was protective in field trials in India.[279] For dengue, many live attenuated strains of all four serotypes are entering phase II efficacy testing. However, none of the vaccines in phase I or II IND status will be available as licensed products soon.

For the remaining VHF agents, availability of effective vaccines is more distant but possible. As with the VHFs noted above, early attempts to develop vaccines against these viruses were based on classical approaches directed primarily at using inactivated

whole virion preparations as vaccines.[280,281] Results from these studies were inconsistent and in general were unsuccessful. Recent VHF vaccine development has been concentrated on various recombinant vectors for expression of VHF-encoded proteins in various combinations to induce protective immunity, and

TABLE 13-2

PREVENTION AND CONTROL OF VIRAL HEMORRHAGIC FEVERS IN HUMANS

Virus Family *Genus*	Disease	Preventive Vaccine	Treatment
Arenaviridae			
Arenavirus	Lassa fever	None	Supportive, Ribavirin[5]
	Argentine HF	IND[1,2]	Supportive, Ribavirin,[6,7] immune plasma[8]
	Bolivian HF	None*	Supportive, Ribavirin,[9] immune plasma[9]
	Brazilian HF	None	Supportive, Ribavirin[10]
	Venezuelan HF	None	Supportive, Ribavirin?
Bunyaviridae			
Nairovirus	Crimean-Congo HF	None	Supportive, Ribavirin[11-13]
Phlebovirus	Rift Valley fever	IND[3]	Supportive, Ribavirin[14]
Hantavirus	HFRS	None†	Supportive, Ribavirin[14,15]
Filoviridae			
Ebolavirus	Ebola HF	None	Supportive, rNAPc2[16]‡
Marburgvirus	Marburg HF	None	Supportive
Flaviviridae			
Flavivirus	Dengue HF	None	Supportive
	Yellow fever	Licensed[4]	Supportive
	Omsk HF	None	Supportive
	Kyasanur forest disease	None	Supportive

*Junin Candid #1 vaccine protects nonhuman primates against Bolivan HF.
†Several vaccines are commercially available in China.
‡A treatment in this case may have some utility but the value is untested and unknown.
HF: hemorrhagic fever
HFRS: hemorrhagic fever with renal syndrome
IND: investigational new drug

Data sources: (1) McKee KT Jr, Barrera-Oro JG, Kuehne AI, Spisso JA, Mahlandt BG. **Candid No. 1 Argentine hemorrhagic fever vaccine** protects against lethal Junin virus challenge in rhesus macaques. *Intervirology.* 1992;34:154–163. **(2) Maiztegui JI, McKee KT Jr, Barrera-Oro JG,** et al. **Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group.** *J Infect Dis.* 1998;177:277–283. (3) **Pittman PR, Liu CT, Cannon TL, et al. Immunogenicity of an inactivated Rift Valley fever vaccine in humans.** *Vaccine.* 1999;18:181–189. (4) Monath TP. Yellow fever vaccine. *Expert Rev Vaccines.* 2005;4:553–574. (5) McCormick JB, King IJ, Webb PA, et al. Lassa fever. Effective therapy with ribavirin. *N Engl J Med.* 1986;314:20–26. (6) Enria DA, Briggiler AM, Levis S, Vallejos D, Maiztegui JI, Canonico PG. Tolerance and antiviral effect of ribavirin in patients with Argentine hemorrhagic fever. *Antiviral Res.* 1987;7:353–359. (7) McKee KT Jr, Huggins JW, Trahan CJ, Mahlandt BG. Ribavirin prophylaxis and therapy for experimental argentine hemorrhagic fever. *Antimicrob Agents Chemother.* 1988;32:1304–1309. (8) **Enria DA, Briggiler AM, Fernandez NJ, Levis SC, Maiztegui JI. Importance of dose of neutralizing antibodies in treatment** of Argentine hemorrhagic fever with immune plasma. *Lancet.* 1984;2:255–256. (9) Kilgore PE, Ksiazek TG, Rollin PE, et al. Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. *Clin Infect Dis.* 1997;24:718–722. (10) Barry M, Russi M, Armstrong L, et al. Brief report: treatment of a laboratory-acquired Sabia virus infection. *N Engl J Med.* 1995;333:294–296. (11) Ergonul O, Celikbas A, Dokuzoguz B, Eren S, Baykam N, Esener H. Characteristics of patients with Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and impact of oral ribavirin therapy. *Clin Infect Dis.* 2004;39:284–287. (12) Fisher-Hoch SP, Khan JA, Rehman S, Mirza S, Khurshid M, McCormick JB. Crimean-Congo hemorrhagic fever treated with oral ribavirin. *Lancet.* 1995;346:472–475. (13) **Mardani M, Jahromi MK, Naieni KH, Zeinali M. The efficacy of oral ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Iran.** *Clin Infect Dis.* 2003;36:1613–1618. (14) Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. *Rev Infect Dis.* 1989;11(suppl 4):S750–S761. (15) Huggins JW, Hsiang CM, Cosgriff TM, et al. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. *J Infect Dis.* 1991;164:1119–1127. (16) **Geisbert TW, Hensley LE,** Jahrling PB, et al. **Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys.** *Lancet.* 2003;362:1953–1958.

tested for protective efficacy in animal models of VHF. Vaccination with recombinant vaccinia viruses expressing Lassa viral proteins successfully protected a majority of cynomolgus and rhesus monkeys from lethal Lassa fever.[177,282] However, a similar strategy using the recombinant vaccinia virus platform as a vaccine for Ebola virus failed to confer any protection to nonhuman primates against lethal Ebola HF.[244] One especially promising strategy has been the use of adenovirus vectors expressing Ebola GP and/or NP genes to protect monkeys against lethal Ebola challenge.[283-285] This platform should be readily adaptable to other HF viruses, and a multivalent VHF vaccine is a plausible possibility. An alternative presentation strategy uses an attenuated vesicular stomatitis virus vector expressing the HF viral glycoprotein of interest. This strategy has successfully protected monkeys against lethal Ebola challenge,[286] Marburg challenge,[286] and Lassa challenges.[287] Other vaccination strategies are under investigation, including virus-like particles[288,289] and alphavirus replicons.[244,290] One technical obstacle to the development of a multivalent VHF vaccine is the potential for prior immunity to the vector, either through natural exposure or prior vaccination, to inhibit immunogenicity.

Postexposure Vaccination

Efforts to develop preventive vaccines against the HF viruses, particularly Ebola, Marburg, and Lassa viruses, have been the most encouraging. Even more encouraging is a result from a recent study in rhesus monkeys showing that the vaccine system based on recombinant vesicular stomatitis virus may not only have utility as a potent preventive vaccine but may also have potential as a postexposure modality.[291] Recombinant vesicular stomatitis virus vectors expressing the Marburg virus glycoprotein were administered to five macaques 20 to 30 minutes after a high-dose lethal injection of homologous Marburg virus. Three animals served as Marburg-positive controls and received nonspecific vectors. All five rhesus monkeys that were treated with the specific Marburg vectors as a postexposure treatment survived a high-dose lethal challenge of Marburg, but all of the control animals developed fulminant disease and died from the Marburg challenge. These results clearly warrant further investigation and potentially provide a new paradigm for treating HF viral infections.

Specific Antiviral Therapy

No antiviral drugs are approved by the US Food and Drug Administration for treating the VHFs. Treatment is primarily by supportive management and palliative care with particular attention given to maintenance of hydration, circulatory volume, blood pressure, and the provision of supplemental oxygen. There is a critical need for the development of effective therapies to respond to outbreaks of VHF in Africa and South America and to counter potential acts of bioterrorism. In addition, the recent death of a Russian scientist after an accidental exposure to Ebola virus[292] emphasizes the need for medical countermeasures for postexposure prophylaxis. Considering the aggressive nature of VHF infections, in particular the rapid and overwhelming viral burdens, early diagnosis plays a significant role in determining the success of any intervention strategy.

Development of effective therapies has been slow for many reasons, including little commercial interest and the need for special containment facilities for safe research. In addition, development of antivirals has been problematic because of the rapid and tremendous increase in viral loads during the acute phase of illness. For example, viremia during the acute phase of Ebola infection of humans or nonhuman primates typically exceeds 6.5 log10 plaque-forming units (pfu)/mL of sera,[25] and in nonhuman primates viremia can go from less than 2.0 log10 pfu/mL to over 5.0 log10 pfu/mL in 24 hours.[114] Thus, a 50% inhibition of virus load may be insignificant in controlling the infection. Additionally, nonhuman primate models indicate that compounds that significantly inhibit Ebola replication in vitro or in rodents[293] may have little efficacy when used in monkeys.[294]

Ribavirin, a nonimmunosuppressive nucleoside analogue with broad antiviral properties,[295] is of proven value for some VHF agents. Ribavirin was shown to reduce mortality from Lassa fever in high-risk patients,[296] and it presumably decreases morbidity in all patients with Lassa fever, for whom current recommendations are to treat initially with ribavirin 30 mg/kg, administered intravenously, followed by 15 mg/kg every 6 hours for 4 days, and then 7.5 mg/kg every 8 hours for an additional 6 days.[273] Treatment is most effective if begun within 7 days of onset; lower intravenous doses or oral administration of 2 g followed by 1 g per day for 10 days also may be useful. Although oral ribavirin is approved by the US Food and Drug Administration for treating chronic hepatitis C virus infection in combination with IFN-α, intravenous ribavirin is of limited availability in the United States. Oral ribavirin is manufactured by ICN Pharmaceuticals Inc (Costa Mesa, Calif) for compassionate use under an IND application.

The primary adverse effects caused by ribavirin have been anemia and hyperbilirubinemia related to a mild hemolysis and reversible block of erythropoiesis. The anemia did not require transfusions or cessation

of therapy in the published Sierra Leone study[296] or in subsequent unpublished limited trials in West Africa. Ribavirin, which is contraindicated in pregnant women, is classified as a pregnancy category X drug.[297] However, in VHF cases of unknown etiology or secondary to an *Arenavirus* or RVF virus, the benefits of treatment are likely to outweigh any fetal risk. Safety of oral or intravenous ribavirin in infants and children has not been established; aerosolized ribavirin has been approved by the Food and Drug Administration to treat respiratory syncytial virus infection in children.

A similar dose of ribavirin initiated within 4 days of disease is efficacious in patients with HFRS.[298,299] In Argentina, ribavirin can reduce virological parameters of Junin virus infection,[300] and is now used routinely as an adjunct to immune plasma. Unfortunately, ribavirin does not penetrate the brain and is expected to protect only against the visceral, and not the neurological, phase of Junin infection.[301]

Small studies investigating the use of ribavirin in treating Bolivian HF and CCHF have been promising,[155,302-304] as have preclinical studies for RVF.[298] Conversely, ribavirin is ineffective against both the filoviruses and the flaviviruses, although a recent study with experimental animals suggests that ribavirin may have some therapeutic utility against yellow fever.[305] Ribavirin is approved for use in treating VHF caused by arenaviruses and bunyaviruses, but not filoviruses, under the compassionate use provisions for INDs. Ribavirin was successfully used to treat a laboratory-acquired Sabia virus infection.[306]

Different preparations of type I IFNs were used in many studies to determine their utility in treating VHFs, with little success.[307-309] At the moment, the type I IFNs appear to have little role in therapy, with the possible exception of RVF, in which fatal HF has been associated with low IFN responses in laboratory animals.[310] Exogenous IFN-γ was also shown to hold promise for treating RVF infections[311]; its role in treating other VHFs is unknown.

Several anti-gene strategies, including approaches based on phosphorodiamidate morpholino oligomers and small interfering RNAs, have been successfully used to protect rodents against Ebola HF[312-314]; however, as mentioned previously, further interest in these strategies is critically dependent on demonstration of postexposure protection in the more stringent nonhuman primate models.

Immunoprophylaxis and Immunotherapy

Passive immunotherapy has been attempted for treating the diseases caused by VHFs owing to the limited availability of effective antiviral drugs. Studies and case reports describing successes and clinical utility[149,315-322] are frequently tempered by more systematic studies, where efficacy is less obvious or of no benefit.[296,323,324] In the case of dengue virus, passively treating rhesus monkeys with antibody to dengue type 2 virus was associated with enhanced dengue type 2 replication.[325] For all HF viruses, the benefit of passive treatment seems to be correlated with the concentration of neutralizing antibodies, which are readily induced by some, but not all, of these viruses.[322,326-328]

Argentine HF responds to antibody therapy with two or more units of convalescent plasma that contain adequate amounts of neutralizing antibody (or an equivalent amount of immune globulin), provided that treatment is initiated within 8 days of onset.[316] Antibody therapy is also beneficial for treating Bolivian HF.[303] Efficacy of immune plasma for treating Lassa fever[327] and CCHF[328] is limited by low neutralizing antibody titers and the consequent need for careful donor selection.

In the future, passive treatment strategies with recombinant human monoclonal antibodies may have utility against the VHF agents given the potential benefit of passive treatment described in many studies.[316,321,322] In HFRS, a passive treatment approach is contraindicated for therapy because an active immune response is usually already evolving in most patients when they are first recognized, although plasma containing neutralizing antibodies has been used empirically in prophylaxis of high-risk exposures.

Modulation of the Host Immune Response

In addition to therapies that are directed toward inhibiting viral replication, strategies to modulate the host response or mitigate the effects of disease may have some utility and should be actively pursued. Two patients infected with Marburg virus in 1975 were given vigorous supportive treatment and prophylactic heparin.[135] This apparent success inspired the use of heparin to treat one of the Ebola patients in the original 1976 outbreak in Zaire[243]; unfortunately, this was unsuccessful. An alternative strategy for Ebola is inhibition of the procoagulant tissue factor pathway. The basis for this speculation is that Ebola virus infection induces overexpression of tissue factor in primate monocytes and macrophages.[239] Based on these data, it was postulated that blocking factor VIIa/tissue factor might be beneficial after Ebola infection.[230] In a preliminary study, nine Ebola-infected monkeys were treated with a protein, recombinant nematode anticoagulant protein c2 (rNAPc2), which prevents blood clotting, and three Ebola-infected monkeys were given a placebo control.[230] Three of the nine treated animals survived, but all three that

were given the placebo control died. In addition, there was a significant delay in death in treated animals that succumbed to the Ebola challenge. Because Ebola infection is nearly 100% fatal in monkeys and kills up to 90% of infected humans, a 33% survival rate for one of the most virulent diseases known is a significant step forward in beginning to develop ways to combat such pathogens. Other study results include the observation that protection of animals was associated with antithrombotic and antiinflammatory effects of the drug, suggesting that strategies that modulate the proinflammatory response may have some therapeutic utility and warrant further investigation.

SUMMARY

During the past decade, extensive coverage has been allocated, in both the popular press and scientific media, to agents causing VHF. Additional information on the VHFs is contained in recent review articles and book chapters.[53,329-332] Some of these viruses may be exploitable as agents of terrorism because they are highly infectious, especially by aerosol, and produce high morbidity and mortality, especially in populations with no prior exposure or herd immunity. Although these viruses vary in their intrinsic attributes and potential use as weapons, all can be introduced into naive populations via natural processes, with fearsome consequences. Increased concern about such natural or unnatural introductions has driven increased investment in basic research and construction of a network of biocontainment laboratories. The dividend will be a more fundamental understanding of the disease processes associated with these infections and identification of potential targets for antiviral drugs, vaccines, and generic countermeasures.

REFERENCES

1. Jahrling PB, Geisbert TW, Dalgard DW, et al. Preliminary report: isolation of Ebola virus from monkeys imported to the USA. *Lancet.* 1990;335:502–505.
2. Bowen MD, Peters CJ, Nichol ST. The phylogeny of New World (Tacaribe complex) arenaviruses. *Virology.* 1996;219:285–290.
3. McCormick JB, Webb PA, Krebs JW, Johnson KM, Smith ES. A prospective study of the epidemiology and ecology of Lassa fever. *J Infect Dis.* 1987;155:437–444.
4. Birmingham K, Kenyon G. Lassa fever is unheralded problem in West Africa. *Nat Med.* 2001;7:878.
5. Freedman DO, Woodall J. Emerging infectious diseases and risk to the traveler. *Med Clin North Am.* 1999;83:865–883.
6. Schmitz H, Kohler B, Laue T, et al. Monitoring of clinical and laboratory data in two cases of imported Lassa fever. *Microbes Infect.* 2002;4:43–50.
7. Centers for Disease Control and Prevention (CDC). Imported Lassa fever – New Jersey, 2004. *MMWR Morb Mortal Wkly Rep.* 2004;53:894–897.
8. Maiztegui J, Feuillade M, Briggiler A. Progressive extension of the endemic area and changing incidence of Argentine Hemorrhagic Fever. *Med Microbiol Immunol.* 1986;175:149–152.
9. Johnson KM, Wiebenga NH, Mackenzie RB, et al. Virus isolations from human cases of hemorrhagic fever in Bolivia. *Proc Soc Exp Biol Med.* 1965;118:113–118.
10. Salas R, de Manzione N, Tesh RB, et al. Venezuelan hemorrhagic fever. *Lancet.* 1991;338:1033–1036.
11. Lisieux T, Coimbra M, Nassar ES, et al. New arenavirus isolated in Brazil. *Lancet.* 1994;343:391–392.
12. Centers for Disease Control and Prevention. Fatal illness associated with a new world arenavirus—California, 1999–2000. *MMWR Morb Mortal Wkly Rep.* 2000;49:709–711.
13. Easterday BC. Rift Valley fever. *Adv Vet Sci.* 1965;10:65–127.

14. Daubney R, Hudson JR, Garnham PC. Enzootic hepatitis or Rift Valley fever: an undescribed virus disease of sheep, cattle and man from East Africa. *J Pathol Bacteriol.* 1931;34:545–579.

15. Shawky S. Rift Valley fever. *Saudi Med J.* 2000;21:1109–1115.

16. van Eeden PJ, van Eeden SF, Joubert JR, King JB, van de Wal BW, Michell WL. A nosocomial outbreak of Crimean-Congo hemorrhagic fever at Tygerberg Hospital, II: management of patients. *S Afr Med J.* 1985;68:718–721.

17. Martinez VP, Bellomo C, San Juan J, et al. Person-to-person transmission of Andes virus. *Emerg Infect Dis.* 2005;11:1848–1853.

18. Nuzum EO, Rossi CA, Stephenson EH, LeDuc JW. Aerosol transmission of Hantaan and related viruses to laboratory rats. *Am J Trop Med Hyg.* 1988;38:636–640.

19. Lee HW. Hemorrhagic fever with renal syndrome in Korea. *Rev Infect Dis.* 1989;11(suppl 4):S864–S876.

20. Butler JC, Peters CJ. Hantaviruses and hantavirus pulmonary syndrome. *Clin Infect Dis.* 1994;19:387–395.

21. Peters CJ, Khan AS. Hantavirus pulmonary syndrome: the new American hemorrhagic fever. *Clin Infect Dis.* 2002;34:1224–1231.

22. Martini GA. Marburg virus disease. Clinical syndrome. In: Martini GA, Siegert R, eds. *Marburg Virus Disease.* New York, NY: Springer-Verlag; 1971: 1–9.

23. Zeller H. Lessons from the Marburg virus epidemic in Durba, Democratic Republic of the Congo (1998–2000) [in French]. *Med Trop (Mars).* 2000;60(suppl 2):23–26.

24. Centers for Disease Control and Prevention (CDC). Outbreak of Marburg virus hemorrhagic fever–Angola, October 1, 2004–March 29, 2005. *MMWR Morb Mortal Wkly Rep.* 2005;54:308–309.

25. World Health Organization International Study Team. Ebola hemorrhagic fever in Zaire, 1976. *Bull World Health Organ.* 1978;56:271–293.

26. World Health Organization International Study Team. Ebola hemorrhagic fever in Sudan, 1976. *Bull World Health Organ.* 1978;56:247–270.

27. Khan AS, Tshioko FK, Heymann DL, et al. The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. *J Infect Dis.* 1999;179(suppl 1):S76–S86.

28. Georges-Courbot MC, Sanchez A, Lu CY, et al. Isolation and phylogenetic characterization of Ebola viruses causing different outbreaks in Gabon. *Emerg Infect Dis.* 1997;3:59–62.

29. Centers for Disease Control and Prevention. Outbreak of Ebola hemorrhagic fever Uganda, August 2000–January 2001. *MMWR Morb Mortal Wkly Rep.* 2001;50:73–77.

30. Walsh PD, Abernethy KA, Bermejo M, et al. Catastrophic ape decline in western equatorial Africa. *Nature.* 2003;422:611–614.

31. Rouquet P, Froment JM, Bermejo M, et al. Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001–2003. *Emerg Infect Dis.* 2005;11:283–290.

32. Le Guenno B, Formenty P, Wyers M, Gounon P, Walker F, Boesch C. Isolation and partial characterization of a new strain of Ebola virus. *Lancet.* 1995;345:1271–1274.

33. Swanepoel R, Leman PA, Burt FJ, et al. Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis.* 1996;2:321–325.

34. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature.* 2005;438:575–576.

35. Monath TP. Yellow fever: Victor, Victoria? Conqueror, conquest? Epidemics and research in the last forty years and prospects for the future. *Am J Trop Med Hyg.* 1991;45:1–43.

36. Pavri K. Clinical, clinicopathological, and hematologic features of Kyasanur forest disease. *Rev Infect Dis.* 1989;11(suppl 4):S854–S859.

37. Chumakov MP. Studies of virus hemorrhagic fevers. *J Hyg Epidemiol Microbiol Immunol.* 1963;7:125–135.

38. Charrel RN, Zaki AM, Attoui H, et al. Complete coding sequence of the Alkhurma virus, a tick-borne flavivirus causing severe hemorrhagic fever in humans in Saudi Arabia. *Biochem Biophys Res Commun.* 2001;287:455–461.

39. Centers for Disease Control and Prevention. Category A Agents. Available at: http://www.bt.cdc.gov/Agent/agentlist.asp. Accessed June 23, 2006.

40. Williams P, Wallace D. *Unit 731, Japan's Secret Biological Warfare in World War II.* New York: The Free Press; 1989: 38–40.

41. Alibek K, Handelman S. *Biohazard: The Chilling True Story of the Largest Covert Biological Weapons Program in the World. Told From the Inside by the Man Who Ran It.* New York, NY: Random House; 1999.

42. Center for Nonproliferation Studies. Chemical and Biological Weapons: Possession and Programs Past and Present. Available at: http://cns.miis.edu/research/cbw/possess.htm. Accessed June 23, 2006.

43. Miller J, Engelberg S, Broad WJ. *Germs: Biological Weapons and America's Secret War.* New York, NY: Simon & Schuster; 2001: 98–123.

44. Bazhutin NB, Belanov EF, Spiridonov VA, et al. The effect of the methods for producing an experimental Marburg virus infection on the characteristics of the course of the disease in green monkeys [in Russian]. *Vopr Virusol.* 1992;37:153–156.

45. P'iankov OV, Sergeev AN, P'iankova OG, Chepurnov AA. Experimental Ebola fever in Macaca mulatta [in Russian]. *Vopr Virusol.* 1995;40:113–115.

46. Johnson E, Jaax N, White J, Jahrling P. Lethal experimental infections of rhesus monkeys by aerosolized Ebola virus. *Int J Exp Pathol.* 1995;76:227–236.

47. Lub MIu, Sergeev AN, P'iankov OV, P'iankova OG, Petrishchenko VA, Kotliarov LA. Certain pathogenetic characteristics of a disease in monkeys infected with the Marburg virus by an airborne route [in Russian]. *Vopr Virusol.* 1995;40:158–161.

48. Stephenson EH, Larson EW, Dominik JW. Effect of environmental factors on aerosol-induced Lassa virus infection. *J Med Virol.* 1984;14:295–303.

49. Kenyon RH, McKee KT Jr, Zack PM, et al. Aerosol infection of rhesus macaques with Junin virus. *Intervirology.* 1992;33:23–31.

50. Global Proliferation of Weapons of Mass Destruction: Hearings Before the Senate Government Affairs Permanent Subcommittee on Investigations, United States Senate, 104th Congress, 1st–2nd Session, 1996. Available at: http://www.fas.org/irp/congress/1995_rpt/aum/part05.htm. Accessed June 23, 2006.

51. Federation of American Scientists. Chemical and Biological Arms Control Program. Weapons of Mass Destruction Around the World. Report on North Korea. Biological Systems. Available at: http://www.fas.org/nuke/guide/dprk/bw/index.html. Accessed June 23, 2006.

52. World Health Organization Group of Consultants. *Health Aspects of Chemical and Biological Weapons.* Geneva, Switzerland: WHO; 1970: 98–109.

53. Borio L, Inglesby T, Peters CJ, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA.* 2002;287:2391–2405.

54. Peters CJ, Jahrling PB, Khan AS. Patients infected with high-hazard viruses: scientific basis for infection control. *Arch Virol Suppl.* 1996;11(Suppl):141–168.

55. International Society for Infectious Diseases. Ebola: stability. February 1, 1997. Archive 19970201.0227. Available at: http://www.promedmail.org. Accessed December 22, 2006.

56. Guzman H, Ding X, Xiao SY, Tesh RB. Duration of infectivity and RNA of Venezuelan equine encephalitis, West Nile, and yellow fever viruses dried on filter paper and maintained at room temperature. *Am J Trop Med Hyg*. 2005;72:474–477.

57. Buchmeier MJ, Bowen MD, Peters CJ. Arenaviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, et al, eds. *Fields Virology.* Philadelphia, Pa: Lippincott Williams & Wilkins; 2001: 1635–1668.

58. Bowen MD, Peters CJ, Nichol ST. The phylogeny of New World (Tacaribe complex) arenaviruses. *Virology.* 1996;219:285–290.

59. Strecker T, Eichler R, Meulen J, et al. Lassa virus Z protein is a matrix protein and sufficient for the release of virus-like particles. *J Virol.* 2003;77:10700–10705.

60. Buchmeier MJ, Southern PJ, Parekh BS, Wooddell MK, Oldstone MB. Site-specific antibodies define a cleavage site conserved among arenavirus GP-C glycoproteins. *J Virol.* 1987;61:982–985.

61. Buchmeier MJ, Lewicki HA, Tomori O, Oldstone MB. Monoclonal antibodies to lymphocytic choriomeningitis and pichinde viruses: generation, characterization, and cross-reactivity with other arenaviruses. *Virology.* 1981;113:73–85.

62. Bruns M, Cihak J, Muller G, Lehmann-Grube F. Lymphocytic choriomeningitis virus. VI. Isolation of a glycoprotein mediating neutralization. *Virology.* 1983;130:247–251.

63. Glushakova SE, Lukashevich IS, Baratova LA. Prediction of arenavirus fusion peptides on the basis of computer analysis of envelope protein sequences. *FEBS Lett.* 1990;269:145–147.

64. Di Simone C, Zandonatti MA, Buchmeier MJ. Acidic pH triggers LCMV membrane fusion activity and conformational change in the glycoprotein spike. *Virology.* 1994;198:455–465.

65. Elliott RM. Molecular biology of the *Bunyaviridae*. *J Gen Virol.* 1990;71:501–522.

66. Bouloy M. *Bunyaviridae*: genome organization and replication strategies. *Adv Virus Res.* 1991;40:235–275.

67. Schmaljohn CS, Hooper JW. *Bunyaviridae*: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, et al, eds. *Fields Virology.* Philadelphia, Pa: Lippincott Williams & Wilkins; 2001: 1581–1602.

68. Weber F, Bridgen A, Fazakerley JK, et al. Bunyamwera bunyavirus nonstructural protein NSs counteracts the induction of alpha/beta interferon. *J Virol.* 2002;76:7949–7955.

69. Sanchez A, Kiley MP, Holloway BP, Auperin DD. Sequence analysis of the Ebola virus genome: organization, genetic elements, and comparison with the genome of Marburg virus. *Virus Res.* 1993;29:215–240.

70. Sanchez A, Khan AS, Zaki SR, Nabel GJ, Ksiazek TG, Peters CJ. *Filoviridae*: Marburg and Ebola viruses. In: Fields BN, Knipe DM, Howley PM, et al, eds. *Fields Virology.* Philadelphia, Pa: Lippincott Williams & Wilkins; 2001.

71. Takada A, Robison C, Goto H, et al. A system for functional analysis of Ebola virus glycoprotein. *Proc Natl Acad Sci U S A.* 1997;94:14764–14769.

72. Ito H, Watanabe S, Sanchez A, Whitt MA, Kawaoka Y. Mutational analysis of the putative fusion domain of Ebola virus glycoprotein. *J Virol.* 1999;73:8907–8912.

73. Sanchez A, Yang ZY, Xu L, Nabel GJ, Crews T, Peters CJ. Biochemical analysis of the secreted and virion glycoproteins of Ebola virus. *J Virol.* 1998;72:6442–6447.

74. Volchkov VE, Feldmann H, Volchkova VA, Klenk H-D. Processing of the Ebola virus glycoprotein by the proprotein convertase furin. *Proc Natl Acad Sci U S A.* 1998;95:5762–5767.

75. Volchkov VE, Becker S, Volchkova VA, et al. GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. *Virology.* 1995;214:421–430.

76. Sanchez A, Trappier SG, Mahy BW, Peters CJ, Nichol ST. The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc Natl Acad Sci U S A.* 1996;93:3602–3607.

77. Noda T, Sagara H, Suzuki E, Takada A, Kida H, Kawaoka Y. Ebola virus VP40 drives the formation of virus-like filamentous particles along with GP. *J Virol.* 2002;76:4855–4865.

78. Han Z, Boshra H, Sunyer JO, Zwiers SH, Paragas J, Harty RN. Biochemical and functional characterization of the Ebola virus VP24 protein: implications for a role in virus assembly and budding. *J Virol.* 2003;77:1793–1800.

79. Chambers TJ, Hahn CS, Galler R, Rice CM. Flavivirus genome organization, expression, and replication. *Annu Rev Microbiol.* 1990;44:649–688.

80. Lindenbach BD, Rice CM. *Flaviviridae*: the viruses and their replication. In: Knipe DM, Howley PM, eds. *Fields Virology.* Philadelphia. Pa: Lippincott Williams & Wilkins; 2001: 991–1041.

81. Mackenzie JM, Jones MK, Young PR. Immunolocalization of the dengue virus nonstructural glycoprotein NS1 suggests a role in viral RNA replication. *Virology.* 1996;220:232–240.

82. Mackenzie JM, Khromykh AA, Jones MK, Westaway EG. Subcellular localization and some biochemical properties of the flavivirus Kunjin nonstructural proteins NS2A and NS4A. *Virology.* 1998;245:203–-215.

83. Liu WJ, Chen HB, Wang XJ, Huang H, Khromykh AA. Analysis of adaptive mutations in Kunjin virus replicon RNA reveals a novel role for the flavivirus nonstructural protein NS2A in inhibition of beta interferon promoter-driven transcription. *J Virol.* 2004;78:12225–12235.

84. Clum S, Ebner KE, Padmanabhan R. Cotranslational membrane insertion of the serine proteinase precursor NS2B-NS3(Pro) of dengue virus type 2 is required for efficient in vitro processing and is mediated through the hydrophobic regions of NS2B. *J Biol Chem.* 1997;272:30715–30723.

85. Gorbalenya AE, Donchenko AP, Koonin EV, Blinov VM. N-terminal domains of putative helicases of flavi- and pestiviruses may be serine proteases. *Nucleic Acids Res.* 1989;17:3889–3897.

86. Preugschat F, Yao CW, Strauss JH. In vitro processing of dengue virus type 2 nonstructural proteins NS2A, NS2B, and NS3. *J Virol.* 1990;64:4364–4374.

87. Arias CF, Preugschat F, Strauss JH. Dengue 2 virus NS2B and NS3 form a stable complex that can cleave NS3 within the helicase domain. *Virology.* 1993;193:888–899.

88. Failla C, Tomei L, De Francesco R. Both NS3 and NS4A are required for proteolytic processing of hepatitis C virus nonstructural proteins. *J Virol.* 1994;68:3753–3760.

89. Lin C, Wu JW, Hsiao K, Su MS. The hepatitis C virus NS4A protein: interactions with the NS4B and NS5A proteins. *J Virol.* 1997;71:6465–6471.

90. Munoz-Jordan JL, Sanchez-Burgos GG, Laurent-Rolle M, Garcia-Sastre A. Inhibition of interferon signaling by dengue virus. *Proc Natl Acad Sci U S A.* 2003;100:14333–14338.

91. Koonin EV. Computer-assisted identification of a putative methyltransferase domain in NS5 protein of flaviviruses and lambda 2 protein of reovirus. *J Gen Virol.* 1993;74:733–740.

92. Tan BH, Fu J, Sugrue RJ, Yap EH, Chan YC, Tan YH. Recombinant dengue type 1 virus NS5 protein expressed in *Escherichia coli* exhibits RNA-dependent RNA polymerase activity. *Virology.* 1996;216:317–325.

93. Halstead SB. Antibody, macrophages, dengue virus infection, shock, and hemorrhage: a pathogenetic cascade. *Rev Infect Dis.* 1989;11(suppl 4):S830–S839.

94. Breman JG, Piot P, Johnson KM, et al. The epidemiology of Ebola hemorrhagic fever in Zaire, 1976. In: Pattyn SR, ed. *Ebola Virus Hemorrhagic Fever.* New York, NY: Elsevier/North-Holland Biomedical Press; 1978: 103–121.

95. McKay DG, Margaretten W. Disseminated intravascular coagulation in virus diseases. *Arch Intern Med.* 1967;120:129–152.

96. Lee HW. Hemorrhagic fever with renal syndrome in Korea. *Rev Infect Dis.* 1989;11(suppl 4):S864–S876.

97. World Health Organization Collaborating Centre for Research and Training in Veterinary Epidemiology and Management. *Report of the WHO/IZSTE Consultation on Recent Developments in Rift Valley Fever (RVF) (With the Participation of FAO and OIE).* 1993;128:1–23. Civitella del Tronto, Italy; 14-15 September 1993. WHO/CDS/VPH/93.123

98. Rosen L. Disease exacerbation caused by sequential dengue infections: myth or reality? *Rev Infect Dis.* 1989;11(suppl 4):S840–S842.

99. Kurane I, Takasaki T. Dengue fever and dengue hemorrhagic fever: challenges of controlling an enemy still at large. *Rev Med Virol.* 2001;11:301–311.

100. Lei HY, Yeh TM, Liu HS, Lin YS, Chen SH, Liu CC. Immunopathogenesis of dengue virus infection. *J Biomed Sci.* 2001;8:377–388.

101. Guzman MG, Kouri G. Dengue: an update. *Lancet Infect Dis.* 2002;2:33–42.

102. Rothman AL. Immunology and immunopathogenesis of dengue disease. *Adv Virus Res.* 2003;60:397–419.

103. Maiztegui JI, Laguens RP, Cossio PM, et al. Ultrastructural and immunohistochemical studies in five cases of Argentine hemorrhagic fever. *J Infect Dis.* 1975;132:35–53.

104. Gonzalez PH, Cossio PM, Arana R, Maiztegui JI, Laguens RP. Lymphatic tissue in Argentine hemorrhagic fever: pathologic features. *Arch Pathol Lab Med.* 1980;104:250–254.

105. Jahrling PB, Hesse RA, Eddy GA, Johnson KM, Callis RT, Stephen EL. Lassa virus infection of rhesus monkeys: pathogenesis and treatment with ribavirin. *J Infect Dis.* 1980;141:580–589.

106. De Brito T, Siqueira SA, Santos RT, Nassar ES, Coimbra TL, Alves VA. Human fatal yellow fever: immunohistochemical detection of viral antigens in the liver, kidney and heart. *Pathol Res Pract.* 1992;188:177–181.

107. Geisbert TW, Jahrling PB, Hanes MA, Zack PM. Association of Ebola-related Reston virus particles and antigen with tissue lesions of monkeys imported to the United States. *J Comp Pathol.* 1992;106:137–152.

108. Zaki SR, Greer PW, Coffield LM, et al. Hantavirus pulmonary syndrome: pathogenesis of an emerging infectious disease. *Am J Pathol.* 1995;146:552–579.

109. Jaax NK, Davis KJ, Geisbert TW, et al. Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. *Arch Pathol Lab Med.* 1996;120:140–155.

110. Burt FJ, Swanepoel R, Shieh WJ, et al. Immunohistochemical and in situ localization of Crimean-Congo hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogenesis. *Arch Pathol Lab Med.* 1997;121:839–846.

111. Geisbert TW, Jaax NK. Marburg hemorrhagic fever: report of a case studied by immunohistochemistry and electron microscopy. *Ultrastruct Pathol.* 1998;22:3–17.

112. Zaki SR, Goldsmith CS. Pathologic features of filovirus infections in humans. *Curr Top Microbiol Immunol.* 1999;235:97–116.

113. de Filippis AM, Nogueira RM, Schatzmayr HG, et al. Outbreak of jaundice and hemorrhagic fever in the Southeast of Brazil in 2001: detection and molecular characterization of yellow fever virus. *J Med Virol.* 2002;68:620–627.

114. Geisbert TW, Hensley LE, Larsen T, et al. Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. *Am J Pathol.* 2003;163:2347–2370.

115. Geisbert TW, Young HA, Jahrling PB, et al. Pathogenesis of Ebola hemorrhagic fever in primate models: evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells. *Am J Pathol.* 2003;163:2371–2382.

116. Monath TP, Barrett AD. Pathogenesis and pathophysiology of yellow fever. *Adv Virus Res.* 2003;60:343–395.

117. Pensiero MN, Sharefkin JB, Dieffenbach CW, Hay J. Hantaan virus infection of human endothelial cells. *J Virol.* 1992;66:5929–5936.

118. Khaiboullina SF, St Jeor SC. Hantavirus immunology. *Viral Immunol.* 2002;15:609–625.

119. Becker S, Spiess M, Klenk HD. The asialoglycoprotein receptor is a potential liver-specific receptor for Marburg virus. *J Gen Virol.* 1995;76:393–399.

120. Chan SY, Empig CJ, Welte FJ, et al. Folate receptor-alpha is a cofactor for cellular entry by Marburg and Ebola viruses. *Cell.* 2001;106:117–126.

121. Alvarez CP, Lasala F, Carrillo J, Muniz O, Corbi AL, Delgado R. C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. *J Virol.* 2002;76:6841–6844.

122. Simmons G, Reeves JD, Grogan CC, et al. DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology.* 2003;305:115–123.

123. Marzi A, Gramberg T, Simmons G, et al. DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. *J Virol.* 2004;78:12090–12095.

124. Takada A, Watanabe S, Ito H, Okazaki K, Kida H, Kawaoka Y. Downregulation of beta1 integrins by Ebola virus glycoprotein: implication for virus entry. *Virology.* 2000;278:20–26.

125. Takada A, Fujioka K, Tsuiji M, et al. Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry. *J Virol.* 2004;78:2943–2947.

126. Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH, Mackow ER. beta3 integrins mediate the cellular entry of hantaviruses that cause respiratory failure. *Proc Natl Acad Sci U S A.* 1998;95:7074–7079.

127. Cao W, Henry MD, Borrow P, et al. Identification of alpha-dystroglycan as a receptor for lymphocytic choriomeningitis virus and Lassa fever virus. *Science.* 1998;282:2079–2081.

128. Spiropoulou CF, Kunz S, Rollin PE, Campbell KP, Oldstone MB. New World arenavirus clade C, but not clade A and B viruses, utilizes alpha-dystroglycan as its major receptor. *J Virol.* 2002;76:5140–5146.

129. Rojek JM, Spiropoulou CF, Kunz S. Characterization of the cellular receptors for the South American hemorrhagic fever viruses Junin, Guanarito, and Machupo. *Virology.* 2006;349:476–491.

130. Gallaher WR, DiSimone C, Buchmeier MJ. The viral transmembrane superfamily: possible divergence of Arenavirus and Filovirus glycoproteins from a common RNA virus ancestor. *BMC Microbiol.* 2001;1:1. Available at: http://www.biomedcentral.com/1471-2180/1/1. Accessed December 22, 2006.

131. Yang Z, Duckers HJ, Sullivan NJ, Sanchez A, Nabel EG, Nabel GJ. Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. *Nat Med.* 2000;6:886–889.

132. Sullivan NJ, Peterson M, Yang ZY, et al. Ebola virus glycoprotein toxicity is mediated by a dynamin-dependent protein-trafficking pathway. *JVirol.* 2005;79:547–553.

133. Alazard-Dany N, Volchkova V, Reynard O, et al. Ebola virus glycoprotein GP is not cytotoxic when expressed constitutively at a moderate level. *J Gen Virol.* 2006;87:1247–1257.

134. Lukashevich IS, Maryankova R, Vladyko AS, et al. Lassa and Mopeia virus replication in human monocytes/macrophages and in endothelial cells: different effects on IL-8 and TNF-alpha gene expression. *J Med Virol.* 1999;59:552–560.

135. Gear JS, Cassel GA, Gear AJ, et al. Outbreak of Marburg virus disease in Johannesburg. *Br Med J.* 1975;4:489–493.

136. Murphy FA. Pathology of Ebola virus infection. In: Pattyn SR, ed. *Ebola Virus Hemorrhagic Fever.* New York, NY: Elsevier/North-Holland Biomedical Press; 1978: 43–59.

137. Feldmann H, Bugany H, Mahner F, Klenk HD, Drenckhahn D, Schnittler HJ. Filovirus-induced endothelial leakage triggered by infected monocytes/macrophages. *J Virol.* 1996;70:2208–2214.

138. Elsner B, Schwarz E, Mando OG, Maiztegui J, Vilches A. Pathology of 12 fatal cases of Argentine hemorrhagic fever. *Am J Trop Med Hyg.* 1973;22:229–236.

139. Terrell TG, Stookey JL, Eddy GA, Kastello MD. Pathology of Bolivian hemorrhagic fever in the rhesus monkey. *Am J Pathol.* 1973;73:477–494.

140. Walker DH, Murphy FA. Pathology and pathogenesis of arenavirus infections. *Curr Top Microbiol Immunol.* 1987;133:89–113.

141. Child PL, MacKenzie RB, Valverde LR, Johnson KM. Bolivian hemorrhagic fever: a pathological description. *Arch Pathol.* 1967;83:434–445.

142. Gedigk P, Bechtelsheimer H, Korb G. Pathologic anatomy of the Marburg virus disease. In: Martini GA, Siegert R, eds. *Marburg Virus Disease.* New York, NY: Springer-Verlag; 1971: 50–53.

143. Winn WC Jr, Walker DH. The pathology of human Lassa fever. *Bull World Health Organ.* 1975;52:535–545.

144. Walker DH, McCormick JB, Johnson KM, et al. Pathologic and virologic study of fatal Lassa fever in man. *Am J Pathol.* 1982;107:349–356.

145. Green DE, Mahlandt BG, McKee KT Jr. Experimental Argentine hemorrhagic fever in rhesus macaques: virus-specific variations in pathology. *J Med Virol.* 1987;22:113–133.

146. Elton NW, Romero A, Trejos A. Clinical pathology of yellow fever. *Am J Clin Pathol.* 1955;25:135–146.

147. Joubert JR, King JB, Rossouw DJ, Cooper R. A nosocomial outbreak of Crimean-Congo hemorrhagic fever at Tygerberg Hospital. Part III. Clinical pathology and pathogenesis. *S Afr Med J.* 1985;68:722–728.

148. Peters CJ, Jahrling PB, Liu CT, Kenyon RH, McKee KT Jr, Barrera-Oro JG. Experimental studies of arenaviral hemorrhagic fevers. *Curr Top Microbiol Immunol.* 1987;134:5–68.

149. Fisher-Hoch SP, McCormick JB, Sasso D, Craven RB. Hematologic dysfunction in Lassa fever. *J Med Virol.* 1988;26:127–135.

150. Peters CJ, Jones D, Trotter R, et al. Experimental Rift Valley fever in rhesus macaques. *Arch Virol.* 1988;99:31–44.

151. Swanepoel R, Gill DE, Shepherd AJ, Leman PA, Mynhardt JH, Harvey S. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev Infect Dis.* 1989;11(suppl 4):S794–S800.

152. Morrill JC, Jennings GB, Johnson AJ, Cosgriff TM, Gibbs PH, Peters CJ. Pathogenesis of Rift Valley fever in rhesus monkeys: role of the interferon response. *Arch Virol.* 1990;110:195–212.

153. Fisher-Hoch SP, Brammer TL, Trappier SG, et al. Pathogenic potential of filoviruses: role of geographic origin of primate host and virus strain. *J Infect Dis.* 1992;166:753–763.

154. Elisaf M, Stefanaki S, Repanti M, Korakis H, Tsianos E, Siamopoulos KC. Liver involvement in hemorrhagic fever with renal syndrome. *J Clin Gastroenterol.* 1993;17:33–37.

155. Ergonul O, Celikbas A, Dokuzoguz B, Eren S, Baykam N, Esener H. Characteristics of patients with Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and impact of oral ribavirin therapy. *Clin Infect Dis.* 2004;39:284–287.

156. Callis RT, Jahrling PB, DePaoli A. Pathology of Lassa virus infection in the rhesus monkey. *Am J Trop Med Hyg.* 1982;31:1038–1045.

157. Frame JD. Clinical features of Lassa fever in Liberia. *Rev Infect Dis.* 1989;11(suppl 4):S783–S789.

158. McLeod CG Jr, Stookey JL, White JD, Eddy GA, Fry GA. Pathology of Bolivian hemorrhagic fever in the African green monkey. *Am J Trop Med Hyg.* 1978;27:822–826.

159. Cosgriff TM. Mechanisms of disease in Hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. *Rev Infect Dis.* 1991;13:97–107.

160. Duchin JS, Koster FT, Peters CJ, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease: the Hantavirus Study Group. *N Engl J Med.* 1994;330:949–955.

161. Peters CJ. Arenaviruses. In: Richman DD, Whitley RJ, Hayden FG, eds. *Clinical Virology.* New York, NY: Churchill Livingstone; 1997: 973–996.

162. Fisher-Hoch SP, Mitchell SW, Sasso DR, Lange JV, Ramsey R, McCormick JB. Physiological and immunologic disturbances associated with shock in a primate model of Lassa fever. *J Infect Dis.* 1987;155:465–474.

163. Hudson N. The pathology of experimental yellow fever in the Macaca rhesus. III. Comparison with the pathology of yellow fever in man. *Am J Pathol.* 1928;4:419–439.

164. Klotz O, Belt TH. Pathology in spleen in yellow fever. *Am J Pathol.* 1930;6:655–662.

165. Zlotnik I. Marburg agent disease: pathology. *Trans R Soc Trop Med Hyg.* 1969;63:310–327.

166. Murphy FA, Simpson DIH, Whitfield SG, Zlotnik I, Carter GB. Marburg virus infection in monkeys: ultrastructural studies. *Lab Invest.* 1971;24:279–291.

167. Edington GM, White HA. The pathology of Lassa fever. *Trans R Soc Trop Med Hyg.* 1972;66:381–389.

168. Monath TP, Brinker KR, Chandler FW, Kemp GE, Cropp CB. Pathophysiologic correlations in a rhesus monkey model of yellow fever with special observations on the acute necrosis of B cell areas of lymphoid tissues. *Am J Trop Med Hyg.* 1981;30:431–443.

169. Baskerville A, Satti A, Murphy FA, Simpson DI. Congo-Crimean hemorrhagic fever in Dubai: histopathological studies. *J Clin Pathol.* 1981;34:871–874.

170. Fisher-Hoch SP, Platt GS, Lloyd G, Simpson DI, Neild GH, Barrett AJ. Hematological and biochemical monitoring of Ebola infection in rhesus monkeys: implications for patient management. *Lancet.* 1983;2:1055–1058.

171. Fisher-Hoch SP, Platt GS, Neild GH, et al. Pathophysiology of shock and hemorrhage in a fulminating viral infection (Ebola). *J Infect Dis.* 1985;152:887–894.

172. McKee KT Jr, Mahlandt BG, Maiztegui JI, Green DE, Peters CJ. Virus-specific factors in experimental Argentine hemorrhagic fever in rhesus macaques. *J Med Virol.* 1987;22:99–111.

173. Simpson DI. Marburg agent disease: in monkeys. *Trans R Soc Trop Med Hyg.* 1969;63:303–309.

174. Havemann K, Schmidt HA. Hematological findings in Marburg virus disease: evidence for involvement of the immunological system. In: Martini GA, Siegert R, eds. *Marburg Virus Disease.* New York, NY: Springer-Verlag; 1971: 34–40.

175. Kastello MD, Eddy GA, Kuehne RW. A rhesus monkey model for the study of Bolivian hemorrhagic fever. *J Infect Dis.* 1976;133:57–62.

176. Wagner FS, Eddy GA, Brand OM. The African green monkey as an alternate primate host for studying Machupo virus infection. *Am J Trop Med Hyg*. 1977;26:159–162.

177. Fisher-Hoch SP, McCormick JB, Auperin D, et al. Protection of rhesus monkeys from fatal Lassa fever by vaccination with a recombinant vaccinia virus containing the Lassa virus glycoprotein gene. *Proc Natl Acad Sci U S A*. 1989;86:317–321.

178. Vallejos DA, Ambrosio AM, Feuillade MR, Maiztegui JI. Lymphocyte subsets alteration in patients with Argentine hemorrhagic fever. *J Med Virol*. 1989;27:160–163.

179. Sanchez A, Lukwiya M, Bausch D, et al. Analysis of human peripheral blood samples from fatal and nonfatal cases of Ebola (Sudan) hemorrhagic fever: cellular responses, virus load, and nitric oxide levels. *J Virol*. 2004;78:10370–10377.

180. Baize S, Leroy EM, Georges-Courbot MC, et al. Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. *Nat Med*. 1999;5:423–426.

181. Geisbert TW, Hensley LE, Gibb TR, Steele KE, Jaax NK, Jahrling PB. Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. *Lab Invest*. 2000;80:171–186.

182. Reed DS, Hensley LE, Geisbert JB, Jahrling PB, Geisbert TW. Depletion of peripheral blood T lymphocytes and NK cells during the course of Ebola hemorrhagic fever in cynomolgus macaques. *Viral Immunol*. 2004;17:390–400.

183. Hensley LE, Young HA, Jahrling PB, Geisbert TW. Proinflammatory response during Ebola virus infection of primate models: possible involvement of the tumor necrosis factor receptor superfamily. *Immunol Lett*. 2002;80:169–179.

184. Mahanty S, Hutchinson K, Agarwal S, McRae M, Rollin PE, Pulendran B. Cutting edge: impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses. *J Immunol*. 2003;170:2797–2801.

185. Bosio CM, Aman MJ, Grogan C, et al. Ebola and Marburg viruses replicate in monocyte-derived dendritic cells without inducing the production of cytokines and full maturation. *J Infect Dis*. 2003;188:1630–1638.

186. Baize S, Kaplon J, Faure C, Pannetier D, Georges-Courbot MC, Deube V. Lassa virus infection of human dendritic cells and macrophages is productive but fails to activate cells. *J Immunol*. 2004;172:2861–2869.

187. Baize S, Leroy EM, Georges AJ, et al. Inflammatory responses in Ebola virus-infected patients. *Clin Exp Immunol*. 2002;128:163–168.

188. Calandra T, Gerain J, Heumann D, Baumgartner JD, Glauser MP. High circulating levels of interleukin-6 in patients with septic shock: evolution during sepsis, prognostic value, and interplay with other cytokines. The Swiss-Dutch J5 Immunoglobulin Study Group. *Am J Med*. 1991;91:23–29.

189. Damas P, Ledoux D, Nys M, et al. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. *Ann Surg*. 1992;215:356–362.

190. Marty C, Misset B, Tamion F, Fitting C, Carlet J, Cavaillon JM. Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin. *Crit Care Med*. 1994;22:673–679.

191. Vermont CL, Hazelzet JA, de Kleijn ED, van den Dobbelsteen GP, de Groot RD. CC and CXC chemokine levels in children with meningococcal sepsis accurately predict mortality and disease severity. *Crit Care*. 2006;10:R33.

192. Levis SC, Saavedra MC, Ceccoli C, et al. Endogenous interferon in Argentine hemorrhagic fever. *J Infect Dis*. 1984;149:428–433.

193. Levis SC, Saavedra MC, Ceccoli C, et al. Correlation between endogenous interferon and the clinical evolution of patients with Argentine hemorrhagic fever. *J Interferon Res*. 1985;5:383–389.

194. Heller MV, Saavedra MC, Falcoff R, Maiztegui JI, Molinas FC. Increased tumor necrosis factor-alpha levels in Argentine hemorrhagic fever. *J Infect Dis*. 1992;166:1203–1204.

195. Groeneveld PH, Colson P, Kwappenberg KM, Clement J. Increased production of nitric oxide in patients infected with the European variant of hantavirus. *Scand J Infect Dis.* 1995;27:453–456.

196. Krakauer T, Leduc JW, Krakauer H. Serum levels of tumor necrosis factor-alpha, interleukin-1, and interleukin-6 in hemorrhagic fever with renal syndrome. *Viral Immunol.* 1995;8:75–99.

197. Marta RF, Montero VS, Hack CE, Sturk A, Maiztegui JI, Molinas FC. Proinflammatory cytokines and elastase-alpha-1-antitrypsin in Argentine hemorrhagic fever. *Am J Trop Med Hyg.* 1999;60:85–89.

198. Mori M, Rothman AL, Kurane I, Montoya JM, Nolte KB, Norman JE. High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. *J Infect Dis.* 1999;179:295–302.

199. Villinger F, Rollin PE, Brar SS, et al. Markedly elevated levels of interferon (IFN)-gamma, IFN-alpha, interleukin (IL)-2, IL-10, and tumor necrosis factor-alpha associated with fatal Ebola virus infection. *J Infect Dis.* 1999;179(suppl 1): S188–S191.

200. Ignatiev GM, Dadaeva AA, Luchko SV, Chepurnov AA. Immune and pathophysiological processes in baboons experimentally infected with Ebola virus adapted to guinea pigs. *Immunol Lett.* 2000;71:131–140.

201. Mahanty S, Bausch DG, Thomas RL, et al. Low levels of interleukin-8 and interferon-inducible protein-10 in serum are associated with fatal infections in acute Lassa fever. *J Infect Dis.* 2001;183:1713–1721.

202. Davis IC, Zajac AJ, Nolte KB, Botten J, Hjelle B, Matalon S. Elevated generation of reactive oxygen/nitrogen species in hantavirus cardiopulmonary syndrome. *J Virol.* 2002;76:8347–8359.

203. ter Meulen J, Sakho M, Koulemou K, et al. Activation of the cytokine network and unfavorable outcome in patients with yellow fever. *J Infect Dis.* 2004;190:1821–1827.

204. Ergonul O, Tuncbilek S, Baykam N, Celikbas A, Dokuzoguz B. Evaluation of serum levels of interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha in patients with Crimean-Congo hemorrhagic fever. *J Infect Dis*. 2006;193:941–944.

205. Papa A, Bino S, Velo E, Harxhi A, Kota M, Antoniadis A. Cytokine levels in Crimean-Congo hemorrhagic fever. *J Clin Virol*. 2006;36:272–276.

206. Stroher U, West E, Bugany H, Klenk HD, Schnittler HJ, Feldmann H. Infection and activation of monocytes by Marburg and Ebola viruses. *J Virol.* 2001;75:11025–11033.

207. Sundstrom JB, McMullan LK, Spiropoulou CF, et al. Hantavirus infection induces the expression of RANTES and IP-10 without causing increased permeability in human lung microvascular endothelial cells. *J Virol.* 2001;75:6070–6085.

208. Geimonen E, Neff S, Raymond T, Kocer SS, Gavrilovskaya IN, Mackow ER. Pathogenic and nonpathogenic hantaviruses differentially regulate endothelial cell responses. *Proc Natl Acad Sci USA*. 2002;99:13837–13842.

209. Gomez RM, Pozner RG, Lazzari MA, et al. Endothelial cell function alteration after Junin virus infection. *Thromb Haemost.* 2003;90:326–333.

210. Khaiboullina SF, Rizvanov AA, Holbrook MR, St Jeor S. Yellow fever virus strains Asibi and 17D-204 infect human umbilical cord endothelial cells and induce novel changes in gene expression. *Virology.* 2005;342:167–176.

211. Leroy EM, Baize S, Volchkov VE, et al. Human asymptomatic Ebola infection and strong inflammatory response. *Lancet.* 2000;355:2210–2215.

212. Peters CJ, Liu CT, Anderson GW Jr, Morrill JC, Jahrling PB. Pathogenesis of viral hemorrhagic fevers: Rift Valley fever and Lassa fever contrasted. *Rev Infect Dis*. 1989;11(suppl 4):S743–S749.

213. Asper M, Sternsdorf T, Hass M, et al. Inhibition of different Lassa virus strains by alpha and gamma interferons and comparison with a less pathogenic arenavirus. *J Virol.* 2004;78:3162–3169.

214. Andersson I, Lundkvist A, Haller O, Mirazimi A. Type I interferon inhibits Crimean-Congo hemorrhagic fever virus in human target cells. *J Med Virol*. 2006;78:216–222.

215. Foster GR, Finter NB. Are all type I human interferons equivalent? *J Viral Hepat*. 1998;5:143–152.

216. Basler CF, Wang X, Muhlberger E, et al. The Ebola virus VP35 protein functions as a type I IFN antagonist. *Proc Natl Acad Sci U S A*. 2000;97:12289–12294.

217. Basler CF, Mikulasova A, Martinez-Sobrido L, et al. The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3. *J Virol*. 2003;77:7945–7956.

218. Basler CF, Palese P. Modulation of innate immunity by filoviruses. In: Klenk HD, Feldmann H, eds. *Ebola and Marburg Viruses: Molecular and Cellular Biology*. Norfolk, United Kingdom: Horizon Bioscience; 2004:305–349.

219. Reid SP, Leung LW, Hartman AL, et al. Ebola virus VP24 binds karyopherin alpha1 and blocks STAT1 nuclear accumulation. *J Virol*. 2006;80:5156–5167.

220. Bouloy M, Janzen C, Vialat P, et al. Genetic evidence for an interferon-antagonist function of Rift Valley fever virus nonstructural protein NSs. *J Virol*. 2001;75:1371–1377.

221. Munoz-Jordan JL, Laurent-Rolle M, Ashour J, et al. Inhibition of alpha/beta interferon signaling by the NS4B protein of flaviviruses. *J Virol*. 2005;79:8004–8013.

222. Li H, Forstermann U. Nitric oxide in the pathogenesis of vascular disease. *J Pathol*. 2000;190:244–254.

223. Moncada S, Higgs EA. The discovery of nitric oxide and its role in vascular biology. *Br J Pharmacol*. 2006;147(suppl 1): S193–S201.

224. Mammen EF. Disseminated intravascular coagulation (DIC). *Clin Lab Sci*. 2000;13:239–245.

225. Levi M. Current understanding of disseminated intravascular coagulation. *Br J Haematol*. 2004;124:567–576.

226. Levi M. Disseminated intravascular coagulation: what's new? *Crit Care Clin*. 2005;21:449–467.

227. Martini GA, Knauff HG, Schmidt HA, Mayer G, Baltzer G. A hitherto unknown infectious disease contracted from monkeys. "Marburg-virus" disease. *Ger Med Mon*. 1968;13:457–470.

228. Bwaka MA, Bonnet MJ, Calain P, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. *J Infect Dis*. 1999;179(suppl 1):S1–S7.

229. Bowen ET, Platt GS, Simpson DI, McArdell LB, Raymond RT. Ebola hemorrhagic fever: experimental infection of monkeys. *Trans R Soc Trop Med Hyg*. 1978;72:188–191.

230. Geisbert TW, Hensley LE, Jahrling PB, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet*. 2003;362:1953–1958.

231. Mackenzie RB, Beye HK, Valverde L, Garron H. Epidemic hemorrhagic fever in Bolivia. I. A preliminary report of the epidemiologic and clinical findings in a new epidemic area in South America. *Am J Trop Med Hyg*. 1964;13:620–625.

232. Peters CJ, Zaki SR, Rollin PE. Viral hemorrhagic fevers. In: Feteky R, ed. *Atlas of Infectious Diseases*. Philadelphia, Pa: Churchill Livingstone; 1997: 10.1–10.26.

233. Molinas FC, de Bracco MM, Maiztegui JI. Coagulation studies in Argentine hemorrhagic fever. *J Infect Dis*. 1981;143:1–6.

234. Dennis LH, Reisberg BE, Crosbie J, Crozier D, Conrad ME. The original hemorrhagic fever: yellow fever. *Br J Haematol*. 1969;17:455–462.

235. Scott SK, Hickman RL, Lang CM, Eddy GA, Hilmas DE, Spertzel RO. Studies of the coagulation system and blood pressure during experimental Bolivian hemorrhagic fever in rhesus monkeys. *Am J Trop Med Hyg*. 1978;27:1232–1239.

236. Molinas FC, Giavedoni E, Frigerio MJ, Calello MA, Barcat JA, Weissenbacher MC. Alteration of blood coagulation and complement system in neotropical primates infected with Junin virus. *J Med Virol*. 1983;12:281–292.

237. Heller MV, Marta RF, Sturk A, et al. Early markers of blood coagulation and fibrinolysis activation in Argentine hemorrhagic fever. *Thromb Haemost*. 1995;73:368–373.

238. Courouble P, Vanpee D, Delgrange E, Donckier J, Pochet JM, Gillet JB. Hantavirus infections: clinical presentation in the emergency room. *Eur J Emerg Med*. 2001;8:17–20.

239. Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, Hensley LE. Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J Infect Dis*. 2003;188:1618–1629.

240. Knobloch J, McCormick JB, Webb PA, Dietrich M, Schumacher HH, Dennis E. Clinical observations in 42 patients with Lassa fever. *Tropenmed Parasitol*. 1980;31:389–398.

241. Lange JV, Mitchell SW, McCormick JB, Walker DH, Evatt BL, Ramsey RR. Kinetic study of platelets and fibrinogen in Lassa virus-infected monkeys and early pathologic events in Mopeia virus-infected monkeys. *Am J Trop Med Hyg*. 1985;34:999–1007.

242. Rippey JJ, Schepers NJ, Gear JH. The pathology of Marburg virus disease. *S Afr Med J*. 1984;66:50–54.

243. Isaacson M, Sureau P, Courteille G, Pattyn SR. Clinical aspects of Ebola virus disease at the Ngaliema Hospital, Kinshasa, Zaire, 1976. In: Pattyn SR, ed. *Ebola Virus Haemorrhagic Fever*. New York, NY: Elsevier/North-Holland Biomedical Press; 1978: 15–20.

244. Geisbert TW, Pushko P, Anderson K, Smith J, Davis KJ, Jahrling PB. Evaluation in nonhuman primates of vaccines against Ebola virus. *Emerg Infect Dis*. 2002;8:503–507.

245. Lee M, Lee JS, Kim BK. Disseminated intravascular coagulation in Korean hemorrhagic fever. *Bibl Haematol*. 1983;49:181–199.

246. Nolte KB, Feddersen RM, Foucar K, et al. Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. *Hum Pathol*. 1995;26:110–120.

247. Santos F, Pereira LC, Paiva M, et al. Coagulacao intravascular diseminada aguda na febre amarela: dosagem dos factores da coagulacao. *Brasilia Med*. 1973;9:9–16.

248. Grignani G, Maiolo A. Cytokines and hemostasis. *Haematologica*. 2000;85:967–972.

249. Neumann F-J, Ott I, Marx N, et al. Effect of human recombinant interleukin-6 and interleukin-8 on monocyte procoagulant activity. *Arterioscler Thromb Vasc Biol*. 1997;17:3399–3405.

250. Szotowski B, Antoniak S, Poller W, Schultheiss HP, Rauch U. Procoagulant soluble tissue factor is released from endothelial cells in response to inflammatory cytokines. *Circ Res*. 2005;96:1233–1239.

251. Ruf W. Emerging roles of tissue factor in viral hemorrhagic fever. *Trends Immunol*. 2004;25:461–464.

252. Volchkov VE, Volchkova VA, Muhlberger E, et al. Recovery of infectious Ebola virus from complementary DNA: RNA editing of the GP gene and viral cytotoxicity. *Science*. 2001;291:1965–1969.

253. Neumann G, Feldmann H, Watanabe S, Lukashevich I, Kawaoka Y. Reverse genetics demonstrates that proteolytic processing of the Ebola virus glycoprotein is not essential for replication in cell culture. *J Virol*. 2002;76:406–410.

254. Enterlein S, Volchkov V, Weik M, et al. Rescue of recombinant Marburg virus from cDNA is dependent on nucleocapsid protein VP30. *J Virol*. 2006;80:1038–1043.

255. Flick R, Flick K, Feldmann H, Elgh F. Reverse genetics for Crimean-Congo hemorrhagic fever virus. *J Virol.* 2003;77:5997–6006.

256. Ikegami T, Won S, Peters CJ, Makino S. Rescue of infectious Rift Valley fever virus entirely from cDNA, analysis of virus lacking the NSs gene, and expression of a foreign gene. *J Virol*. 2006;80:2933–2940.

257. Bredenbeek PJ, Kooi EA, Lindenbach B, Huijkman N, Rice CM, Spaan WJ. A stable full-length yellow fever virus cDNA clone and the role of conserved RNA elements in flavivirus replication. *J Gen Virol.* 2003;84:1261–1268.

258. Rubins KH, Hensley LE, Jahrling PB, et al. The host response to smallpox: analysis of the gene expression program in peripheral blood cells in a nonhuman primate model. *Proc Natl Acad Sci U S A.* 2004;101:15190–15195.

259. Kash JC, Muhlberger E, Carter V, et al. Global suppression of the host antiviral response by Ebola and Marburg viruses: increased antagonism of the type I interferon response is associated with enhanced virulence. *J Virol*. 2006;80:3009–3020.

260. Centers for Disease Control and Prevention, National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. Washington, DC: Government Printing Office; 1999. Available at: http://www.cdc.gov/od/ohs/pdffiles/4th%20BMBL.pdf. Accessed June 23, 2006.

261. van der Groen G, Elliot LH. Use of betapropiolactone inactivated Ebola, Marburg and Lassa intracellular antigens in immunofluorescent antibody assay. *Ann Soc Belg Med Trop.* 1982;62:49–54.

262. Trappier SG, Conaty AL, Farrar BB, Auperin DD, McCormick JB, Fisher-Hoch SP. Evaluation of the polymerase chain reaction for diagnosis of Lassa virus infection. *Am J Trop Med Hyg.* 1993;49:214–221.

263. Leroy EM, Baize S, Lu CY, et al. Diagnosis of Ebola hemorrhagic fever by RT-PCR in an epidemic setting. *J Med Virol.* 2000;60:463–467.

264. Drosten C, Gottig S, Schilling S, et al. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *J Clin Microbiol.* 2002;40:2323–2330.

265. Towner JS, Rollin PE, Bausch DG, et al. Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. *J Virol*. 2004;78:4330–4341.

266. Grolla A, Lucht A, Dick D, Strong JE, Feldmann H. Laboratory diagnosis of Ebola and Marburg hemorrhagic fever. *Bull Soc Pathol Exot*. 2005;98:205–209.

267. Palacios G, Briese T, Kapoor V, et al. MassTag polymerase chain reaction for differential diagnosis of viral hemorrhagic fever. *Emerg Infect Dis*. 2006;12:692–695.

268. Geisbert TW, Rhoderick JB, Jahrling PB. Rapid identification of Ebola virus and related filoviruses in fluid specimens using indirect immunoelectron microscopy. *J Clin Pathol.* 1991;44:521–522.

269. Geisbert TW, Jahrling PB. Differentiation of filoviruses by electron microscopy. *Virus Res.* 1995;39:129–150.

270. Zaki SR, Shieh WJ, Greer PW, et al. A novel immunohistochemical assay for the detection of Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. Commission de Lutte contre les Epidemies a Kikwit. *J Infect Dis.* 1999;179(suppl 1):S36–S47.

271. Levi M, de Jonge E, van der Poll T. Plasma and plasma components in the management of disseminated intravascular coagulation. *Best Pract Res Clin Haematol*.2006;19:127–142.

272. Fisher-Hoch SP. Arenavirus pathophysiology. In: Salvato MS, ed. *The Arenaviridae.* New York, NY: Plenum Press; 1993: 299–323.

273. Centers for Disease Control. Management of patients with suspected viral hemorrhagic fever. *MMWR Morb Mortal Wkly Rep.* 1988;37(suppl 3):1–16.

274. Monath TP. Yellow fever vaccine. *Expert Rev Vaccines*. 2005;4:553–574.

275. McKee KT Jr, Barrera-Oro JG, Kuehne AI, Spisso JA, Mahlandt BG. Candid No. 1 Argentine hemorrhagic fever vaccine protects against lethal Junin virus challenge in rhesus macaques. *Intervirology.* 1992;34:154–163.

276. Maiztegui JI, McKee KT Jr, Barrera-Oro JG, et al. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. *J Infect Dis.* 1998;177:277–283.

277. Pittman PR, Liu CT, Cannon TL, et al. Immunogenicity of an inactivated Rift Valley fever vaccine in humans. *Vaccine.* 1999;18:181–189.

278. Hooper JW, Li D. Vaccines against hantaviruses. *Curr Top Microbiol Immunol*. 2001;256:171–191.

279. Dandawate CN, Desai GB, Achar TR, Banerjee K. Field evaluation of formalin inactivated Kyasanur forest disease virus tissue culture vaccine in three districts of Karnataka state. *Indian J Med Res.* 1994;99:152–158.

280. Geisbert TW, Jahrling PB. Towards a vaccine against Ebola virus. *Expert Rev Vaccines.* 2003;2:777–789.

281. Fisher-Hoch SP, McCormick JB. Lassa fever vaccine. *Expert Rev Vaccines.* 2004;3:189–197.

282. Fisher-Hoch SP, Hutwagner L, Brown B, McCormick JB. Effective vaccine for Lassa fever. *J Virol.* 2000;74:6777–6783.

283. Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. *Nature.* 2000;408:605–609.

284. Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebola virus hemorrhagic fever in nonhuman primates. *Nature.* 2003;424:681–684.

285. Sullivan NJ, Geisbert TW, Geisbert JB, et al. Immune protection of nonhuman primates against Ebola virus with single low-dose adenovirus vectors encoding modified GPs. *PLos Med*. 2006;3:e177.

286. Jones SM, Feldmann H, Stroher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med.* 2005;11:786–790.

287. Geisbert TW, Jones S, Fritz EA, et al. Development of a new vaccine for the prevention of Lassa fever. *PLoS Med.* 2005;2: e183. Epub 2005 June 28.

288. Warfield KL, Bosio CM, Welcher BC, et al. Ebola virus-like particles protect from lethal Ebola virus infection. *Proc Natl Acad Sci U S A.* 2003;100:15889–15894.

289. Warfield KL, Swenson DL, Demmin G, Bavari S. Filovirus-like particles as vaccines and discovery tools. *Expert Rev Vaccines*. 2005;4:429–440.

290. Hevey M, Negley D, Pushko P, Smith J, Schmaljohn A. Marburg virus vaccines based upon alphavirus replicons protect guinea pigs and nonhuman primates. *Virology.* 1998;251:28–37.

291. Daddario-DiCaprio KM, Geisbert TW, Stroher U, et al. Postexposure protection against Marburg hemorrhagic fever with recombinant vesicular stomatitis virus vectors in nonhuman primates: an efficacy assessment. *Lancet*. 2006;367:1399–1404.

292. International Society for Infectious Diseases. Ebola, Lab Accident Death - Russia (Siberia), May 22, 2004. Archive 20040522.1377. Available at: http://www.promedmail.org. Accessed December 20, 2006.

293. Huggins J, Zhang ZX, Bray M. Antiviral drug therapy of filovirus infections: S-adenosylhomocysteine hydrolase inhibitors inhibit Ebola virus in vitro and in a lethal mouse model. *J Infect Dis.* 1999;179(suppl 1):S240–S247.

294. Huggins JW, Zhang ZX, Davis K, Coulombe RA. Inhibition of Ebola virus by S-adenosylhomocysteine hydrolase inhibitors. *Antiviral Res.* 1995;26:301.

295. Canonico PG, Kende M, Luscri BJ, Huggins JW. In-vivo activity of antivirals against exotic RNA viral infections. *J Antimicrob Chemother.* 1984;14(suppl A):27–41.

296. McCormick JB, King IJ, Webb PA, et al. Lassa fever. Effective therapy with ribavirin. *N Engl J Med.* 1986;314:20–26.

297. Rebetol product information. Available at: http://www.spfiles.com/pirebetol.pdf. Accessed June 23, 2006.

298. Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. *Rev Infect Dis.* 1989;11(suppl 4):S750–S761.

299. Huggins JW, Hsiang CM, Cosgriff TM, et al. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. *J Infect Dis*. 1991;164:1119–1127.

300. Enria DA, Briggiler AM, Levis S, Vallejos D, Maiztegui JI, Canonico PG. Tolerance and antiviral effect of ribavirin in patients with Argentine hemorrhagic fever. *Antiviral Res.* 1987;7:353–359.

301. McKee KT Jr, Huggins JW, Trahan CJ, Mahlandt BG. Ribavirin prophylaxis and therapy for experimental argentine hemorrhagic fever. *Antimicrob Agents Chemother*. 1988;32:1304–1309.

302. Fisher-Hoch SP, Khan JA, Rehman S, Mirza S, Khurshid M, McCormick JB. Crimean-Congo hemorrhagic fever treated with oral ribavirin. *Lancet.* 1995;346:472–475.

303. Kilgore PE, Ksiazek TG, Rollin PE, et al. Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. *Clin Infect Dis.* 1997;24:718–722.

304. Mardani M, Jahromi MK, Naieni KH, Zeinali M. The efficacy of oral ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Iran. *Clin Infect Dis.* 2003;36:1613–1618.

305. Sbrana E, Xiao SY, Guzman H, Ye M, Travassos da Rosa AP, Tesh RB. Efficacy of postexposure treatment of yellow fever with ribavirin in a hamster model of the disease. *Am J Trop Med Hyg.* 2004;71:306–312.

306. Barry M, Russi M, Armstrong L, et al. Brief report: treatment of a laboratory-acquired Sabia virus infection. *N Engl J Med.* 1995;333:294–296.

307. Ignat'ev GM, Strel'tsova MA, Agafonov AP, Kashentseva EA, Prozorovskii NS. Experimental study of possible treatment of Marburg hemorrhagic fever with desferal, ribavirin, and homologous interferon [in Russian]. *Vopr Virusol.* 1996;41:206–209.

308. Jahrling PB, Geisbert TW, Geisbert JB, et al. Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infections. *J Infect Dis.* 1999;179(suppl 1):S224–S234.

309. Kolokol'tsov AA, Davidovich IA, Strel'tsova MA, Nesterov AE, Agafonova OA, Agafonov AP. The use of interferon for emergency prophylaxis of Marburg hemorrhagic fever in monkeys. *Bull Exp Biol Med*.2001;132:686–688.

310. Morrill JC, Jennings GB, Cosgriff TM, Gibbs PH, Peters CJ. Prevention of Rift Valley fever in rhesus monkeys with interferon-alpha. *Rev Infect Dis.* 1989;11(suppl 4):S815–S825.

311. Morrill JC, Czarniecki CW, Peters CJ. Recombinant human interferon-gamma modulates Rift Valley fever virus infection in the rhesus monkey. *J Interferon Res.* 1991;11:297–304.

312. Warfield KL, Swenson DL, Olinger GG, et al. Gene-specific countermeasures against Ebola virus based on antisense phosphorodiamidate morpholino oligomers. *PLos Pathog*. 2006;2:e1. Epub January 13.

313. Enterlein S, Warfield KL, Swenson DL, et al. VP35 knockdown inhibits Ebola virus amplification and protects against lethal infection in mice. *Antimicrob Agents Chemother*. 2006;50:984–993.

314. Geisbert TW, Hensley LE, Kagan E, et al. Postexposure protection of guinea pigs against a lethal Ebola virus challenge is conferred by RNA interference. *J Infect Dis.* 2006;193:1650–1657.

315. Emond RT, Evans B, Bowen ET, Lloyd G. A case of Ebola virus infection. *Br Med J.* 1977;2:541–544.

316. Enria DA, Briggiler AM, Fernandez NJ, Levis SC, Maiztegui JI. Importance of dose of neutralizing antibodies in treatment of Argentine hemorrhagic fever with immune plasma. *Lancet.* 1984;2:255–256.

317. Monath TP, Maher M, Casals J, Kissling RE, Cacciapuoti A. Lassa fever in the Eastern Province of Sierra Leone, 1970–1972. II. Clinical observations and virological studies on selected hospital cases. *Am J Trop Med Hyg.* 1974;23:1140–1149.

318. Mupapa K, Massamba M, Kibadi K, et al. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. *J Infect Dis.* 1999;179(suppl 1):S18–S23.

319. Stille W, Bohle E, Helm E, van Rey W, Siede W. On an infectious disease transmitted by Cercopithecus aethiops ("Green monkey disease") [in German]. *Dtsch Med Wochenschr.* 1968;93:572–582.

320. Leifer E, Gocke DJ, Bourne H. Lassa fever, a new virus disease of man from West Africa. II. Report of a laboratory-acquired infection treated with plasma from a person recently recovered from the disease. *Am J Trop Med Hyg.* 1970;19:677–679.

321. Frame JD, Verbrugge GP, Gill RG, Pinneo L. The use of Lassa fever convalescent plasma in Nigeria. *Trans R Soc Trop Med Hyg.* 1984;78:319–324.

322. Maiztegui JI, Fernandez NJ, de Damilano AJ. Efficacy of immune plasma in treatment of Argentine hemorrhagic fever and association between treatment and a late neurological syndrome. *Lancet.* 1979;2:1216–1217.

323. White HA. Lassa fever: a study of 23 hospital cases. *Trans R Soc Trop Med Hyg.* 1972;66:390–401.

324. Clayton AJ. Lassa immune serum. *Bull World Health Organ.* 1977;55:435–439.

325. Halstead SB. In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *J Infect Dis.* 1979;140:527–533.

326. Jahrling PB, Peters CJ. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infect Immun.* 1984;44:528–533.

327. Jahrling PB, Frame JD, Rhoderick JB, Monson MH. Endemic Lassa fever in Liberia. IV. Selection of optimally effective plasma for treatment by passive immunization. *Trans R Soc Trop Med Hyg.* 1985;79:380–384.

328. Shepherd AJ, Swanepoel R, Leman PA. Antibody response in Crimean-Congo hemorrhagic fever. *Rev Infect Dis.* 1989;11(suppl 4):S801–S806.

329. Schnittler HJ, Feldmann H. Viral hemorrhagic fever: a vascular disease? *Thromb Haemost.* 2003;89:967–972.

330. Geisbert TW, Marty AM, Jahrling PB. Viral hemorrhagic fevers. In: Roy MJ, ed. *Physician's Guide to Terrorist Attack.* Totowa, NJ: Humana Press; 2004: 221–242.

331. Geisbert TW, Jahrling PB. Exotic emerging viral diseases: progress and challenges. *Nat Med.* 2004;10(suppl 12): S110–121.

332. Bray M. Pathogenesis of viral hemorrhagic fever. *Curr Opin Immunol.* 2005;17:399–403.