The Pathogenesis of Ebola Virus Disease*

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Abstract

For almost 50 years, ebolaviruses and related filoviruses have been repeatedly reemerging across the vast equatorial belt of the African continent to cause epidemics of highly fatal hemorrhagic fever. The 2013–2015 West African epidemic, by far the most geographically extensive, most fatal, and longest lasting epidemic in Ebola's history, presented an enormous international public health challenge, but it also provided insights into Ebola's pathogenesis and natural history, clinical expression, treatment, prevention, and control. Growing understanding of ebolavirus pathogenetic mechanisms and important new clinical observations of the disease course provide fresh clues about prevention and treatment approaches. Although viral cytopathology and immune-mediated cell damage in ebolavirus disease often result in severe compromise of multiple organs, tissue repair and organ function recovery can be expected if patients receive supportive care with fluids and electrolytes; maintenance of oxygenation and tissue perfusion; and respiratory, renal, and cardiovascular support. Major challenges for managing future Ebola epidemics include establishment of early and aggressive epidemic control and earlier and better patient care and treatment in remote, resource-poor areas where Ebola typically reemerges. In addition, it will be important to further develop Ebola vaccines and to adopt policies for their use in epidemic and pre-epidemic situations.

INTRODUCTION

EBOV: Ebola virus

One of the deadliest of epidemic viral diseases, Ebola virus disease (EVD) has, since its 1976 emergence, challenged our attempts to understand its ecology and epidemiology, its pathophysiology and pathogenesis, and its optimal treatment and prevention. The 2013–2015 West African epidemic (1), by far the largest in Ebola's history, provides a new opportunity to understand this important disease in ways that can potentially lead to significant reduction in mortality in the future.

In this review, we address the pathogenesis, pathology, and pathophysiology of ebolaviruses in relation to clinical observations and opportunities for clinical intervention, with the specific aim of stimulating thought about developing better treatment approaches not only in Western Intensive Care Unit (ICU) settings but, more importantly, in resource-limited African settings with fewer supportive care options. Emphasis is placed on Ebola virus (EBOV) representing the species *Zaire ebolavirus* because most of our knowledge about ebolaviruses is derived from studying this virus and the outbreaks it has caused.

HISTORICAL PERSPECTIVES

The sudden 1976 emergence of ebolaviruses in proximate areas of Zaire (now the Democratic Republic of the Congo) and Sudan (about 850 kilometers away from the Zaire epidemic) (2) shocked medical science, attracted worldwide attention, and terrified people in the epidemic area. Immediately recognized as a new viral hemorrhagic fever. EVD was added to an accelerating list of other viral hemorrhagic fevers such as yellow fever (known for centuries); Crimean-Congo hemorrhagic fever (first recorded in 1944); Korean hemorrhagic fever (1951); dengue hemorrhagic fever (1953); and Argentinian, Bolivian, and Lassa (arenavirus) hemorrhagic fevers (1955–1969). These diverse agents, emerging from different reservoirs, exhibiting different modes of transmission, and having different natural histories and pathogeneses, all feature severe systemic viral infection associated with one or more hemorrhagic phenomena such as petechiae, ecchymoses, or frank bleeding.

Although it was originally assumed that the two proximate 1976 ebolavirus outbreaks were a single event, in which one or the other area had been seeded by the arrival of an infected traveler, investigations discovered that the epidemics were caused by two phylogenetically related but genetically distinct ebolaviruses (3, 4), *Zaire ebolavirus* and *Sudan ebolavirus*. Why had two novel viruses emerged at the same time in adjacent locales? To virologists and epidemiologists, the most plausible answer was emergence from two different reservoirs (e.g., two different mammals) under the influence of some common factor, both viruses then independently spilling over into humans. This explanation was based on findings from many other sylvatic viruses indicating that viral reservoir hosts are typically species to which a virus becomes stably adapted over long periods of time, causing minimal or no disease in that host and being maintained via silent circulation within the host species either by host-to-host transmission or via intermediate hosts or vectors.

In the years following the 1976 ebolavirus outbreaks, trapping and bleeding of thousands of animals and insects failed to identify a reservoir (5, 6). It later became apparent that gorillas, chimpanzees, and other mammals suffered ebolavirus emergences and epizootics, but only as dead-end hosts who became infected, as humans did, when the virus emerged from its unknown reservoir(s). Over the past 30 years, fieldwork has clarified that ebolaviruses may be distributed in sylvatic ecosystems across the African tropical belt, prominently involving several species of fruit bats, which have been speculated to be either reservoir hosts themselves or involved in ebolavirus emergence in some other way. This discussion emphasizes EBOV representing the species *Zaire ebolavirus* because of its greater public health and medical significance, and because a much larger

body of research has been published about this virus than about other ebolaviruses. With the exception of members of the species *Reston ebolavirus*, which appear to be nonpathogenic for humans, evidence does not suggest significant differences in human pathology or pathogenesis between the ebolaviruses. The filovirus family has recently been comprehensively reviewed (7, 8).

SUDV: Sudan virus

MARV: Marburg virus

VIROLOGY

Phylogenetic Classification

The family *Filoviridae*, of the order *Mononegavirales*, is comprised of three genera, *Ebolavirus*, *Marburgvirus*, and *Cuevavirus* (**http://www.ictvonline.org/virustaxonomy.asp**; 9); the genus *Ebolavirus* is further subdivided into five species, each of which is represented by a unique type virus: *Taï Forest ebolavirus* (Taï Forest virus, TAFV), *Reston ebolavirus* (Reston virus, RESTV), *Sudan ebolavirus* (Sudan virus, SUDV), *Bundibugyo ebolavirus* (Bundibugyo virus, BDBV), and *Zaire ebolavirus* (Ebola virus, EBOV).

There are multiple EBOV isolates, including EBOV-Mayinga, the historical laboratory reference strain derived from the 1976 outbreak, and EBOV-Makona, the strain that circulated in West Africa from 2013–2015. Marburg virus (MARV) and Ravn virus (RAVV) are closely related type viruses of the single recognized species *Marburg marburgvirus* in the genus *Marburgvirus*; Lloviu virus (LLOV) is the type virus of the species *Lloviu cuevavirus* in the genus *Cuevavirus*, but cuevaviruses have yet to be isolated (10).

Viral Biology

EBOV is a nonsegmented negative-strand RNA virus with a genome size of approximately 19 kb. The genome displays the following structure: 3′-leader → *nucleoprotein (NP)* gene → *viral protein (VP) 35* gene → *VP40* gene → *glycoprotein (GP)* gene → *VP30* gene → *VP24* gene → *polymerase (L)* gene → 5′-trailer. The conserved leader and trailer regions contain genome replication promoters and packaging signals. Each gene is flanked by 3′ and 5′ untranslated regions (UTRs) including conserved transcriptional start and stop signals. Most genes are separated by intergenic regions of variable lengths, although some genes overlap in parts of their UTRs. All genes are monocistronic with the exception of *GP*, which encodes a total of three glycoproteins. The primary product of *GP* is the soluble GP (sGP). Through RNA editing performed by the viral polymerase complex, ebolaviruses downregulate expression of the transmembrane GP and produce a second small soluble glycoprotein (ssGP). Such RNA editing is a feature that distinguishes the ebolaviruses from the marburgviruses (8, 9).

EBOV particles (**Figure 1**) have a uniform diameter of about 80 nm but vary greatly in length (up to 14,000 nm) and form, with the filamentous form being the most characteristic. Both of the soluble glycoproteins are nonstructural proteins and are secreted from infected cells. The core structure of filovirus particles is composed of the RNA genome, NP, VP35, VP30, and L., and is further associated with VP24. VP40 is the membrane-associated matrix protein; it forms the filamentous structure of viral particles and connects the nucleocapsid with the host-derived lipid membrane. The carboxyl-terminal end of GP is inserted into the membrane as a type I transmembrane glycoprotein (8, 11).

EBOV proteins have distinct functions in the replication cycle (8, 9, 11, 12). NP encapsidates the genome, protects the RNA from degradation, and is a major component of the viral ribonucleoprotein complex (RNP). VP35, a polymerase cofactor, is a phosphoprotein equivalent of other mononegavirales. VP30 is a transcriptional activator supporting primary transcription as

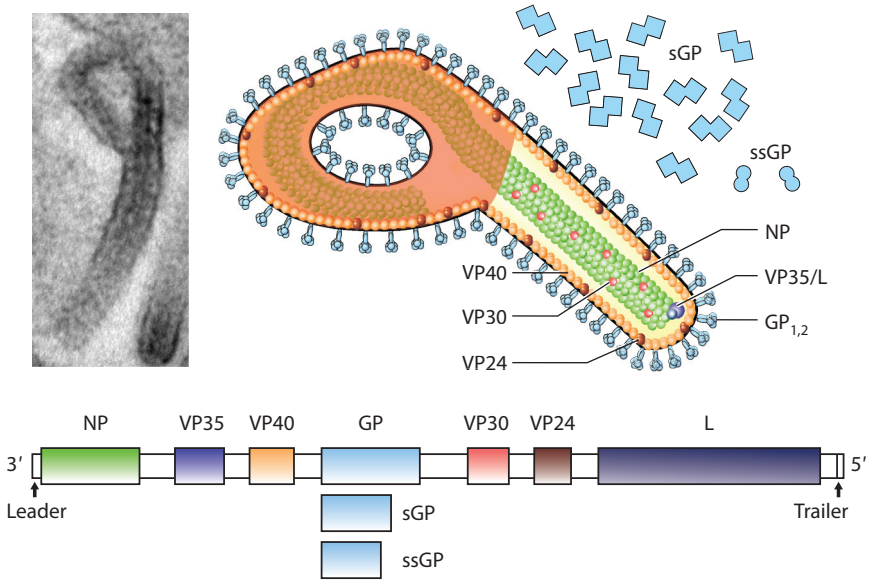

Figure 1

Ebolavirus structure indicating various proteins and the genes that code for them. The genome displays the following structure: 3′-leader → *nucleoprotein (NP)* gene → *viral protein (VP) 35* gene → *VP40* gene → *glycoprotein (GP)* gene → *VP30* gene → *VP24* gene → *polymerase (L)* gene → 5′-trailer.

well as RNA editing. The RNA-dependent RNA polymerase L carries the enzymatic functional domains for genome transcription and replication. These four viral proteins form the RNP and are sufficient to drive genome replication and transcription. VP24 plays an important role in RNP assembly, and VP40 mediates the budding of particles from infected cells. GP mediates virus entry through receptor binding and fusion within the endosome.

EBOV infects cells by attaching to a variety of cellular attachment molecules, including lectin-type molecules. Virus particles enter cells by macropinocytosis and, to a lesser degree, by clathrin-mediated endocytosis. The surface GP undergoes proteolytic processing (i.e., by cathepsins) in the endosome, allowing it to interact with the cellular receptor cholesterol transporter Niemann-Pick C1 (NPC1) protein, to fuse with the endosomal membrane, and to release the RNP into the cytoplasm, where primary transcription is initiated. This is followed by secondary transcription and viral genome replication, the latter of which occurs in so-called inclusion bodies. New viral genomes are then transported to the cell surface in the form of nucleocapsids and packaged together with plasma membrane–inserted GP into progeny viral particles, a process that is mediated by VP40 (11, 13).

EBOV VP35 and VP24 are, respectively, strong inhibitors of interferon production and of interferon signaling. VP35 has multiple additional functions that interfere with the early innate host response (12, 14, 15). The functions of sGP and ssGP remain unknown. sGP has been thought to act as a decoy for antibodies that otherwise would bind to GP (8, 16).

Viral Evolution

All known EBOV isolates are related to but genetically distinct from each other. Phylogenetic evidence has satisfactorily established neither the antiquity of these viruses nor the mechanisms

and pathways by which they evolved into geographically distinct isolates. Evidence that exists is incomplete and in some cases contradictory (17–20), but viral and epidemiologic data appear consistent with the possibility that the ebolaviruses have long existed in some unknown reservoir or reservoirs and that their recent emergences have much to do with human factors such as population size and movement, bushmeat hunting practices and bushmeat consumption, and other variables associated with human activities. It is of interest that during the recent West African epidemic, which featured intense, prolonged chains of person-to-person transmission at a level never before observed, the virus evolved by sequential adaptation without any evidence of significant phenotypic change (17, 21).

EPIDEMIOLOGY AND ECOLOGY

Viral Reservoir

EBOV reservoirs have been sought for 40 years with little definitive success. The geographic ranges of many animal species, including bats, squirrels, mice and rats, dormice, and shrews, match or overlap with known outbreak sites of African filoviruses (6), but none of these mammals has yet been universally accepted as an EBOV reservoir. Serologic studies have often shown low EBOV seropositivity rates in people and various animals before, during, and after epidemics. However, the serologic tests typically used in these studies are known to often give false positive results. The circulation of EBOV in nature and the mechanisms of its reemergence may be complex and may reflect unappreciated ecological dynamics (22, 23).

EBOV epidemics have been temporally associated with bat migrations, infections, and die-offs (although fruit bats, among the prime reservoir suspects, apparently do not develop filovirus disease). Nevertheless, attempts to implicate bats as EBOV reservoirs have been problematic. In both epidemic and postepidemic periods, attempts to identify animal (including bat) reservoirs have been hampered by inability, with a few exceptions, to isolate EBOV or detect viral RNA sequences. Possible explanations for this failure include very low prevalence of infection or transient EBOV infection in animals, virus infection at very low titer, or unknown variables associated with virus reactivation from a state of latency (18, 24).

Bat antibody to ebolaviruses is largely absent or at low prevalence until the time that epidemics or epizootics occur (25), and bat seropositivity rates have generally not been well correlated with epidemic or epizootic occurrences (26). Although a variety of filoviruses appear to enter the migratory bat population from time to time, often in a transient manner, it has not yet been possible to satisfactorily link these phenomena to establishment of a reservoir for human and mammalian spillover. However, the association of a new cuevavirus with European bats (10) and a stronger case for bats, in particular *Rousettus aegyptiacus*, serving as reservoirs of Marburg viruses (27) add greatly to the plausibility of bats serving as EBOV reservoirs. The ultimate source of Ebola viruses in nature and the mechanisms and pathways by which they emerge to infect humans remain a mystery that significantly impedes the development of prevention and control approaches. The complicated case for implicating bats as EBOV reservoirs has been well reviewed (7, 25, 26, 28–30).

Geographic Distribution

Ebolavirus outbreaks have occurred across the entire equatorial belt of Africa, especially along riparian systems, with EBOV outbreaks being most common in western Central and West Africa and SUDV and BDBV outbreaks being most common in eastern Central Africa (**Figure 2**). Researchers have developed maps of geographical areas believed to be at high risk of future

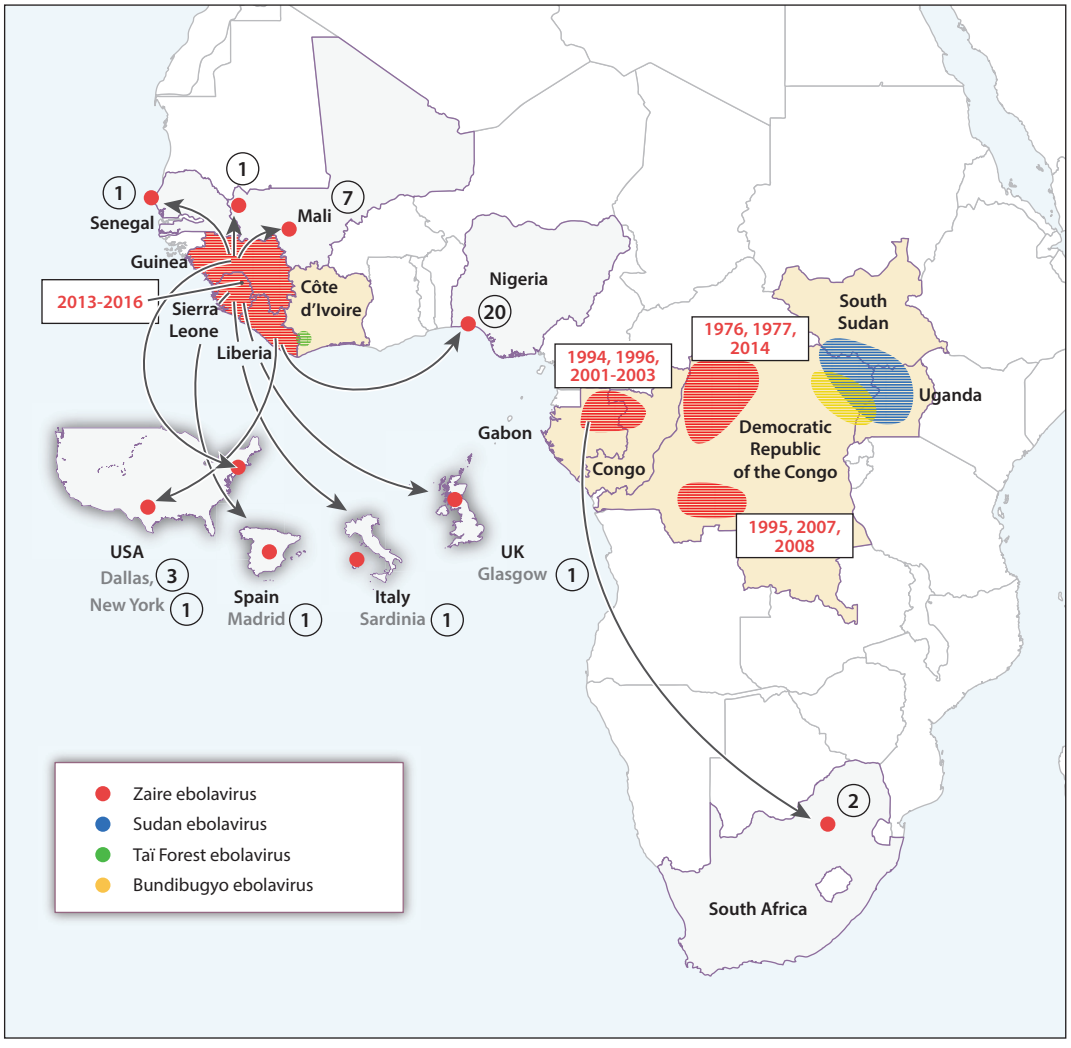

Figure 2

Geographic locales of known ebolavirus outbreaks in West and Central Africa (from 1976 to August 2016). The map shows the occurrence of all known human outbreaks associated with four different ebolavirus species across the equatorial belt within the African continent. The circled numbers indicate EVD cases resulting from introductions of EBOV-infected persons from Guinea, Sierra Leone, Liberia, or, in one instance, from an individual infected in Gabon during the 2013–2015 West African epidemic.

ebolavirus outbreaks (31), but the use of these maps may be limited by the enormity of the areas identified, which span 22 countries inhabited by 22 million people. Since the two 1976 epidemics, there have been an additional 23 ebolavirus epidemics, of which fourteen have been caused by EBOV, six by SUDV, two by BDBV, and a single case by TAFV (32).

TAFV: Taï Forest virus

Outside of Africa, Reston virus (RESTV) has been found in Southeast Asia but, despite proven human infections, has not been associated with disease (8). Cuevavirus sequences have been detected in Spanish bats but association with human disease is unknown. The fact that phylogenetically distinct ebolaviruses are also geographically restricted is consistent with the possible

existence of multiple distinct reservoirs associated with either different host species or a single species existing in different isolated ecosystems.

Incidence and Prevalence

Basic epidemiologic features of EVD are known almost entirely from investigations of explosive circumscribed or multifocal outbreaks associated with hospital spread, person-to-person community spread, or both. EBOV affects humans of all ages, from infancy to old age, including live-born children of infected mothers (2). Age-specific incidence rates rise linearly from early childhood, leveling off in the 35–44-year-old age range and decreasing slightly thereafter (33). Lower attack rates in children and older adults and higher attack rates in women presumably reflect differences in exposures associated with caregiving for ill persons. In some epidemics, index or early cases have been hunters or others with wild animal exposures (2).

As was the case during the initial 1976 outbreaks (2), residents of affected villages usually claim that EVD had previously been unknown in the area (with the notable exception of EBOV outbreaks in the border region of Gabon and the Republic of the Congo). Because EBOV reemergences tend to produce intense focal outbreaks in noncontiguous villages, disease incidence rates calculated over standard (large) geographic areas may be low. However, within the outbreak area there may be hospitals or small villages with very high attack rates, reaching up to 76% (2). In the 1976 epidemic and in most recent epidemics, low percentages of people living in the general epidemic area who did not become ill were subsequently found to have EBOV antibody (2), often in the 2.5–5% range, a percentage similar to the seroprevalence rate of EVD contacts who do not exhibit clinical illnesses (2); in at least one study, this latter figure was estimated to be significantly higher (34). Possible explanations for such seropositive individuals include nonspecificity of the serologic assays employed, inherent resistance to infection or to progression of infection, silent infection due to low dose or inefficient route of exposure, or exposure to unidentified serologically cross-reacting filoviruses.

Case–Fatality Ratio

The case–fatality ratios in the 1976 epidemics were 88% for EBOV and about 60% for SUDV (2). In the 1995 Kikwit EBOV outbreak, the ratio was little changed at 81% (35), and it has tended to remain at a similarly high level whenever outbreaks occur in remote locations with limited access to advanced medical care. It was surprising that case–fatality ratios in the recent West African EBOV epidemic tended to fall in the 40% range, a possible reflection of the efficacy of basic supportive care given in Ebola Treatment Units (ETUs) and other more advanced medical facilities and of earlier case detection, admission, and initiation of supportive care. Other possible explanations for which evidence might be sought include decreased virulence of the emergent strain or attenuation during prolonged human-to-human transmission.

Mode of Transmission

The route of primary transmission from the unknown reservoir to an end host species such as humans remains unknown. Human-to-human EBOV transmission occurs via inoculation by injection of virus into the bloodstream or via exposure of mucous membranes or nonintact skin to infectious body fluids or tissues. Direct (versus indirect) contact with infectious material is associated with significantly increased risk of infection. Infectious viral particles in proteinaceous material survive on inanimate surfaces for days or weeks and can survive even longer in conditions

such as climate-controlled hospital settings (36), allowing previously contaminated fomites to serve as viable sources of infection.

The 1976 EBOV epidemic was associated primarily with hospital transmission via contaminated needles or syringes. In a much smaller number of persons (about 5%), infection was explained only by very close contact exposure to ill persons (2). Such persons presumably acquired infection via skin breaks or mucosal inoculation (8). Subsequent epidemics have been associated with one or more of these modes of transmission, as well as with community person-to-person superspreaders (35). In the 2014–2015 Sierra Leone epidemic, 74% of transmission events were between family or extended family members, 18% between friends and other community contacts, and 8% within hospitals (37). Secondary (nonparenteral) contact case rates rarely exceeded 10–15% (8). The mean incubation period in those 1976 cases with documented injection exposure was 6.3 days (2). A more common incubation period in other epidemics associated with person-to-person spread has been around 9–10 days (37).

Investigations have repeatedly indicated that secondary cases without injection exposures are usually a result of intimate exposure through caregiving or burial preparation, especially when exposure involves contact with infectious body fluids (38). The recent West African epidemic featured long chains of intensive community person-to-person transmission associated with both intimate and unappreciated exposures. A meta-analysis estimated that the risk of secondary transmission within households of an acutely ill EBOV-infected patient was <1% for persons who did not provide any care for the patient; 2.1% for all household contacts combined, regardless of care provided; 22.9% for those having any type of direct patient contact; and 47.9% for those providing direct care (34). A different study (39) estimated an 83% attack rate for persons touching corpses. One of the most dangerous mechanisms of EBOV spread seems to be body washing and other burial practices (40). The institution of safer burial preparation practices has been credited with epidemic control (41). Although aerosol transmission of EBOV and SUDV have been reported in a rhesus macaque challenge experiment (42) and in other experimental challenges (43–46), careful epidemiologic and clinical studies have not supported a critical role for airborne spread in human outbreaks.

PATHOGENESIS AND PATHOLOGY

In humans and in nonhuman primates (NHPs), high-level EBOV replication associated with systemic dissemination to multiple cell types results in a complex pathogenesis that includes both detrimental immune suppression and immune overactivation in different aspects of the immune response, disordered coagulation, and tissue damage due to direct viral and indirect host-mediated effectors. In the absence of adequate supportive care, these processes commonly result in multiple organ failure and death within about 10 days of symptom onset in humans. **Figure 3** outlines the pathogenesis of EBOV infection.

Viral Dissemination and Cellular Tropism

Following viral inoculation onto mucous membranes or nonintact skin, EBOV targets dendritic cells and other cells of the monocyte/macrophage lineage (47). These virus-infected cells then travel via lymphatic vessels to lymph nodes and nodal chains, where virus replication and dissemination occur prior to the onset of symptoms. In the first 3 days after symptom onset, estimation of viral load by quantitative reverse transcriptase polymerase chain reaction (q-RT PCR) assay increases exponentially in blood, from undetectable levels to often $>10^5$ viral particles/ml (48, 49), consistent with the virus overcoming innate immune control mechanisms to become widely

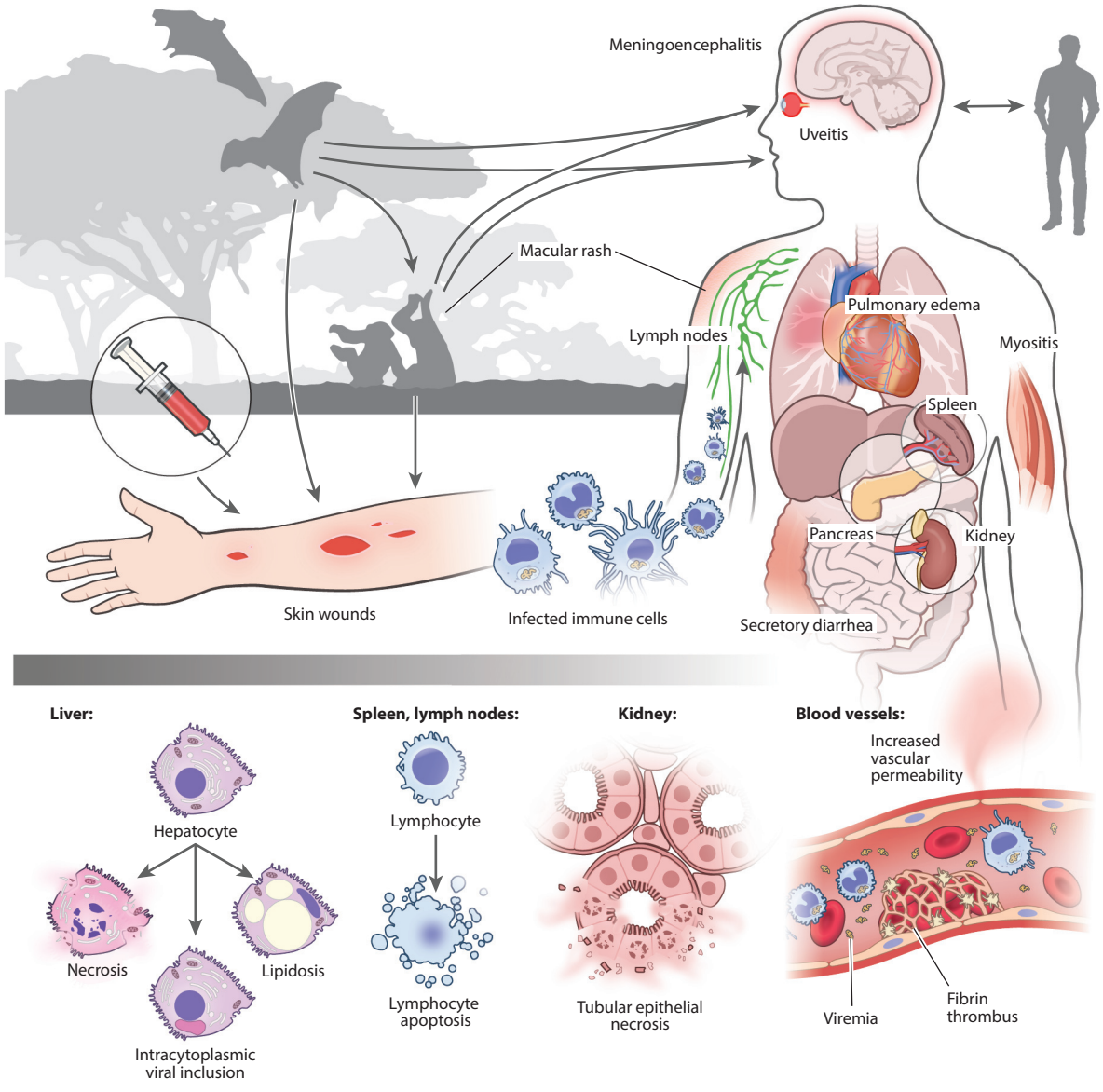

Figure 3

The transmission and pathogenesis of ebolavirus infection. Zoonotic, nosocomial, or person-to-person transmission of ebolavirus leads to viral infection of mononuclear phagocytes, which transport the virus to regional lymph nodes. Virus replication is followed by viremia with widespread viral dissemination, leading to tissue and vascular damage.

disseminated via the bloodstream (50). Human viral load determination, i.e., titration of infectious virions, has not been systematically studied, although high viremia during illness has been documented. It is unfortunate that, for such a highly fatal and highly transmissible infection, investigation of the recent West African epidemic has not yet provided more complete data comparing viral RNA detection by PCR with virus titers in blood and body fluids associated with infection transmission. Early in the infectious process, multiple organs, particularly the liver and spleen,

Table 1 Mediators of ebolavirus immune evasion

Viral mediator	Major mechanism of immune evasion	References
VP24	Inhibits type I IFN signaling	(55, 56, 58, 59)
VP35	Inhibits type I IFN production	(53, 60–62)
	Inhibits dendritic cell maturation	(52, 64)
sGP	Binds anti-GP neutralizing antibodies	(65)

Abbreviations: GP, glycoprotein; IFN, interferon; sGP, soluble glycoprotein; VP, viral protein.

and multiple cell types within these organs become infected. Lymphocytes appear to be a rare cell type that escapes viral infection (51).

Evasion of the Immune System

Early extensive EBOV replication and dissemination is predicated upon effective evasion of host immune responses. EBOV employs multiple mechanisms to achieve this (**Table 1**), including antagonism of the type I interferon (IFN) response, mediated by EBOV structural proteins VP24 and VP35 (52–57). VP24 blocks IFN signaling by preventing the dimerization of tyrosine kinases and nuclear translocation of signal transducer and activator of transcription 1 (STAT1) (55, 58, 59). The IFN inhibitory domain (IID) of VP35 binds dsRNA in host cells and prevents it from binding the cytoplasmic receptors *retinoic acid-inducible gene-I* (*RIG-I*) and *melanoma differentiation-associated gene 5* (*MDA-5*). This impairs phosphorylation of IFN regulatory factor (IRF)-3 and IRF-7, thereby inhibiting type I IFN expression (53, 60–63). Furthermore, VP35 inhibits maturation of dendritic cells by interfering with the RIG-I signaling pathway to prevent upregulation of MHC I and MHC II and the costimulatory molecules CD40, CD80, and CD86, thus impairing antigen presentation to CD8+ and CD4+ T cells and T-cell activation (52, 64) and thereby impeding linkage of the innate and adaptive immune responses.

sGP, a viral protein released in abundance during acute illness and thus circulating at high levels in the serum, acts as a decoy by binding EBOV-neutralizing antibodies to impair a protective humoral immune response (65). It is hypothesized that glycosylation of transmembrane GP may sterically impede the binding of neutralizing antibodies to it (66), as is the case with a number of unrelated viruses. Although lymphocytes are not infected by EBOV, lymphocyte apoptosis results in early lymphopenia and lymphoid depletion in the spleen and lymph nodes, further affecting adaptive immune responses (67–69). The mechanism underlying lymphocyte apoptosis is not well understood, although intrinsic and extrinsic apoptotic pathways are both thought to be involved (70–73). Rasmussen (74) has comprehensively reviewed the multiple host factors that play a role in human EBOV infections.

Pathology

Although thousands of people have died from EVD, few autopsies or biopsies have been performed, and those that have are largely limited to cases from early outbreaks. Lesions detected at autopsy include petechiae and ecchymoses in the mucous membranes and parenchymatous organs, hemorrhage in the gastrointestinal tract lumen, congestion of abdominal organs, and hepatomegaly and splenomegaly (68, 75–77). In the liver, hepatocellular necrosis with minimal inflammation, the primary histologic finding, may occur in conjunction with congestion, mild periportal inflammation, Kupffer cell hypertrophy and hyperplasia, microvesicular lipidosis, mild

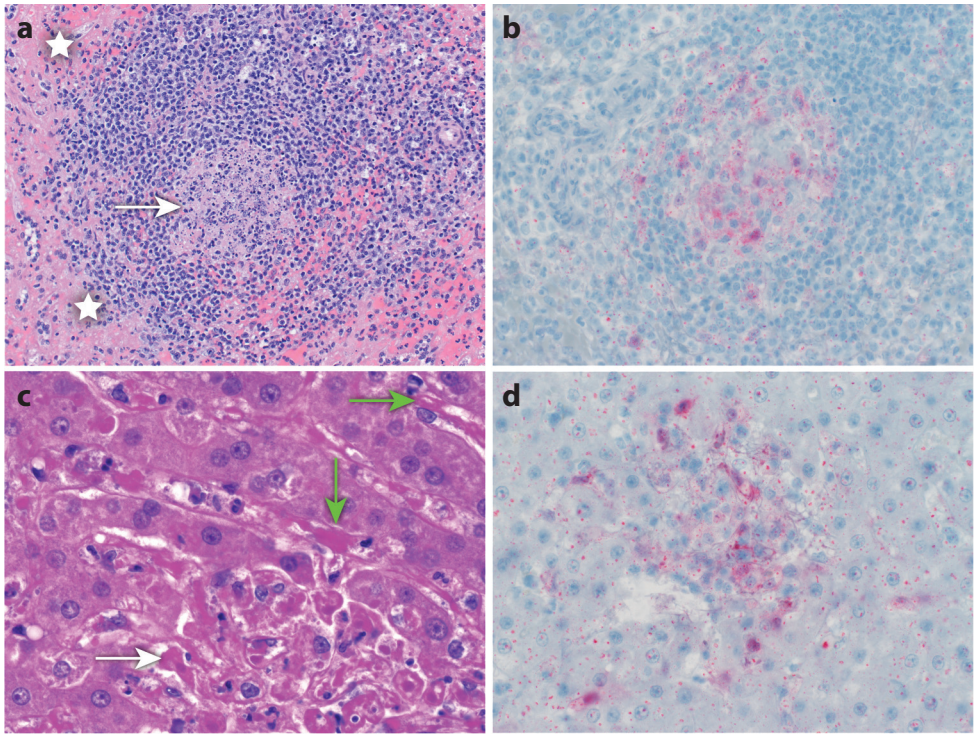

Figure 4

EBOV pathology in experimental animals. Sections of spleen and liver from rhesus macaques intramuscularly inoculated with EBOV were stained with hematoxylin and eosin (H&E) (*a* and *c*) or were labeled with anti-EBOV antibody using immunohistochemistry (IHC) (*b* and *d*). (*a*) In the spleen, there is lymphoid depletion with replacement by necrotic cellular debris (*arrow*) and fibrin deposition within the red pulp (*stars*). (*b*) Viral antigen (*labeled in red*) is present among the necrotic cellular debris in the splenic white pulp. (*c*) In the liver, there is hepatocellular necrosis (*white arrow*) and degeneration; multiple hepatic sinusoids are occluded by fibrin thrombi (*green arrows*). (*d*) Viral antigen (*labeled in red*) is observed within hepatocytes.

cholestasis, and formation of Councilman bodies (51, 68, 75, 76, 78, 79). Eosinophilic intracytoplasmic viral inclusions are frequently observed in hepatocytes (51, 76, 79, 80), and viral antigen is commonly detected in hepatocytes, Kupffer cells, and portal tracts (51, 68).

Microscopic lesions in the spleen and lymph nodes are characterized by lymphocyte apoptosis leading to lymphoid depletion and accumulation of cellular debris (68, 75, 78) (**Figure 4**). In splenic lesions, viral antigen has been identified in dendritic cells, fibroblasts, and macrophages (68, 76). Cutaneous biopsies show dermal edema, hemorrhage, epidermal spongiosis, vascular lesions such as endothelial degeneration and necrosis, and infrequent fibrin thrombi (51, 76). Viral antigen is present in endothelial cells, dendritic cells, fibroblasts, and sweat and sebaceous glands in the skin (51, 76).

Lesions have also been described in the digestive tract, lung, kidney, heart, and bone marrow. Despite the presence of hemorrhage in the gastrointestinal tract at autopsy, only mild inflammation in the lamina propria, in conjunction with the presence of viral antigen in infiltrating mononuclear leukocytes, has been identified microscopically (51, 77). Alveolar edema, hemorrhage, and congestion with minimal to no inflammation have been reported in the lungs; viral antigen is found in alveolar macrophages (68). Renal tubular epithelial necrosis and degeneration

with interstitial edema and mild inflammation, hemorrhage, and congestion have been reported in the kidneys; both tubular epithelium and endothelium may become infected by EBOV (51, 68, 75, 78, 81). Myocardial edema with little to no inflammation has rarely been reported, and viral antigen has been detected in endocardial and endothelial cells (68). Bone marrow is reportedly normocellular with focal necrosis; viral inclusions are observed in mononuclear cells, and extracellular EBOV antigen has been detected in the bone marrow by immunohistochemistry (IHC) (51).

The presence of hyperamylasemia in some patients suggests acute pancreatitis; however, histologic features of pancreatitis have not been described in human EBOV infections (82). Adrenocortical cell necrosis has been reported in animal models of EBOV infection (47, 58, 83), although the clinical correlate in humans is not clear. Although lesions have not been reported in skeletal muscle, the presence of myalgia and high levels of creatine phosphokinase suggest rhabdomyositis with or without rhabdomyolysis (84).

Late onset of ocular abnormalities, including panuveitis, vitritis, iridocyclitis, and intraretinal hemorrhages, has been observed in some EVD survivors (85, 86). Because EBOV has been isolated from the aqueous humor of an affected eye in a nonviremic survivor, ocular lesions are thought to be caused by persistence of EBOV in this immune-privileged tissue (85). Late-onset orchitis has been rarely reported in survivors. Both viral RNA and infectious virus have been detected in semen, and cases of suspected male-to-female sexual transmission have been reported, suggesting that the testis is another immune-privileged tissue targeted by EBOV (81, 87, 88). In support of this possibility, fibrin thrombi have been observed microscopically in the testes, and viral antigen has been detected within such thrombi, in endothelial cells, and in the seminiferous tubules (51).

Mechanisms of Tissue Damage

Tissue damage in EBOV-infected individuals is caused by multiple interrelated mechanisms, including but not limited to direct viral-induced cytopathic effects and indirect organ injury mediated by host inflammatory responses, endothelial dysfunction, and disordered coagulation (**Table 2**). In vitro studies have shown that cytopathic effects, including cell rounding and detachment, are directly mediated by viral GP (89, 90). IHC has shown colocalization of tissue necrosis and viral antigen in fatal cases of human EVD (76). Proinflammatory cytokines and chemokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, and MIP-1β, as well as increased TNF-related apoptosis-inducing ligand (TRAIL) expression and production of nitric oxide (NO), have been

Table 2 Major mediators of ebolavirus tissue damage

Mediator	Major mechanism of tissue damage	References
Proinflammatory cytokines, chemokines	Recruit leukocytes → recruited NK cells and CD8+ T cells mediate cytotoxicity	(92, 93)
Nitric oxide	Induces cell apoptosis or necrosis	(94, 95)
Tissue factor	Activates extrinsic coagulation pathway → fibrin thrombus formation → ischemic necrosis of tissues	(97)
Increased TRAIL expression or Fas–FasL interactions	Induces lymphocyte apoptosis	(70–72)
EBOV GP	Directly mediates cytopathic effects	(89, 90)

Abbreviations: EBOV, Ebola virus; GP, glycoprotein; NK, natural killer; TRAIL, tumor necrosis factor–related apoptosis-inducing ligand.

variably observed in fatal EVD and likely contribute to organ injury (67, 70, 73, 91). Proinflammatory cytokines and chemokines recruit leukocytes to infected tissues in order to limit virus dissemination and kill virus-infected host cells, thus contributing to tissue damage (92, 93). NO released by activated macrophages may trigger parenchymal cell apoptosis or necrosis (94, 95). In vitro studies suggest that increased TRAIL expression and increased Fas–Fas ligand interactions can trigger lymphocyte apoptosis, leading to severe lymphoid depletion in the spleen and lymph nodes (71).

EBOV infection of mononuclear phagocytes and immune system activation contribute to increased tissue factor expression on monocytes/macrophages (69, 96). Tissue factor triggers the extrinsic coagulation pathway leading to fibrin thrombus formation (97). TNF-α may decrease thrombomodulin levels, and liver injury may impair production of proteins C and S; these effects jointly contribute to decreased anticoagulant effects (94, 98, 99). Coagulopathy in EVD is characterized by thrombocytopenia, formation and deposition of fibrin thrombi, and increased fibrin degradation products (47, 69, 76, 78, 82, 83). Microvascular fibrin thrombi contribute to tissue ischemia and necrosis; clotting factor consumption predisposes the infected individual to bleeding within tissues and from mucosal surfaces (69, 100). Classical signs of EVD, including conjunctival and gingival hemorrhages and hemorrhages from body orifices and venipuncture sites, are thought to represent disseminated intravascular coagulation (DIC) (68, 75, 76).

EBOV also infects endothelial cells; swelling, necrosis, and loss of endothelial cells have been reported in a few fatal cases and likely contribute to vascular permeability and intravascular coagulation (51, 76). In vitro, EBOV GP causes endothelial cell rounding, detachment, and loss, potentially compromising intercellular tight junctions and leading to increased vascular permeability (89, 90). Release of NO by endothelial cells and monocytes/macrophages, and of TNF-α by monocytes/macrophages, induces vasodilation and increased vascular permeability, thus contributing to intravascular volume depletion (101, 102).

Animal Models

Animal models of EVD have been important for identifying early target cells of infection and organ pathology; characterizing inflammatory responses, vascular dysfunction, and coagulation abnormalities; and evaluating potential routes of transmission. Furthermore, animal models have allowed for evaluation of the efficacy of vaccines and therapeutics.

Mice. Multiple mouse models of EBOV infection have been described; however, even the most promising of these models fails to fully mimic human EVD. Wild-type EBOV does not cause disease in adult immunocompetent mice (103) but can cause delayed disease in immunocompromised mice (104). Serial passage of EBOV is required to produce a mouse-adapted EBOV (MA-EBOV) capable of causing fatalities in adult immunocompetent mice (103). Acquired mutations in VP24 and NP in MA-EBOV are thought to cause the increased pathogenicity (105). Intraperitoneal (IP) inoculation of mice with MA-EBOV produces several features of human EVD, including viral targeting of the spleen and liver; hepatocellular degeneration and necrosis; elevated liver enzymes; and mild coagulopathy characterized by thrombocytopenia, occasionally accompanied by prolonged clotting times (103, 106). Other features of human disease, such as fever, rash, prominent bystander lymphocyte apoptosis, marked deposition of fibrin in the spleen, and DIC are not consistently seen in mice (103, 106, 107). However, EBOV susceptibility and pathogenicity appear to be influenced by the genetic background of mice, as evidenced by the development of multiple recombinant inbred mouse strains that are highly susceptible to fatal MA-EBOV infections that are accompanied by a coagulopathy (107).

Guinea pigs. In guinea pigs, adaptation by serial passage of EBOV is also required for production of consistently lethal infections (108). Subcutaneous inoculation of inbred guinea pigs or IP inoculation of outbred guinea pigs with guinea pig–adapted EBOV (GP-EBOV) causes fever, weight loss, neutrophilia, leukocytosis, lymphopenia, increased liver enzymes, and increased blood urea nitrogen (BUN) and creatinine, signs similar to those of human EVD (109, 110). Additionally, increased proinflammatory cytokine levels, bystander apoptosis of lymphocytes in lymphoid organs, and severe coagulation abnormalities occur in infected outbred guinea pigs (109). Adrenal cortical necrosis and hepatocellular degeneration have been reported (109, 110). Councilman bodies have been detected in hepatocytes, and intracytoplasmic viral inclusions have been identified in both adrenal cortical cells and hepatocytes (109, 110). Viremia and high viral titers are detected in the liver, spleen, and adrenal glands (106, 110). Although many features of human EVD are observed in guinea pigs, the characteristic rash is lacking in both inbred and outbred guinea pigs, and few to no hemorrhages are grossly visible in inbred guinea pigs (110). Additionally, granulomatous foci have been described in the liver of guinea pigs (111) but not humans; the significance of this lesion has yet to be determined.

Syrian hamsters. As in other rodent models, wild-type EBOV causes minimal changes in Syrian hamsters. IP inoculation with wild-type EBOV causes a subclinical infection, a weak and transient increase in proinflammatory cytokines, and mild inflammation in multiple organs (112). However, IP inoculation with MA-EBOV causes uniformly lethal disease in Syrian hamsters that models human EVD more accurately than the mouse model does (112). In Syrian hamsters, MA-EBOV targets the liver, spleen, and lymph nodes, causing microscopic lesions reminiscent of those in humans, including hepatocellular necrosis and widespread lymphoid depletion attributed to apoptosis (112). Severe coagulopathy suggestive of DIC, characterized by thrombocytopenia, decreased protein C, prolonged clotting times, and hypofibrinogenemia, is also reported in Syrian hamsters (112). However, in contrast to humans, hamsters show less fibrin accumulation in the liver and spleen and do not develop a rash (112).

Nonhuman primates (NHPs). EVD has been studied in rhesus macaques, cynomolgus macaques, African green monkeys, baboons, and common marmosets (47, 113–115); each of these species substantially reproduces prominent features of human EVD. Currently, macaque models are considered the gold standard for experimental EVD due to their consistent similarity to human disease. In NHPs, fever, diarrhea, and rash often develop, as do typical leukogram and biochemistry panel changes (47, 113–115). Increases in NO, and the proinflammatory cytokines IL-6, TNF-α, and IFN-γ, have also been detected (47, 73). Similar cell types and organs are targeted in humans and NHPs, resulting in comparable hepatic and lymphoid organ lesions (47, 113–115). Additionally, NHPs develop DIC characterized by prolonged coagulation times, decreased protein C levels, increased D-dimer levels, and increased tissue factor expression (47, 114, 115). These disease characteristics have been described after inoculation through different routes, including intramuscular, IP, subcutaneous, oral, conjunctival, or aerosol inoculation (45, 47, 113, 114, 116).

NHPs are currently the only animals in which ocular pathology associated with EBOV antigen, similar to that in humans, has been documented after recovery from EVD (85, 86, 117). Additionally, because both lesions and EBOV antigen have been found in the testes of infected NHPs, they may be useful in modeling viral infections of the male reproductive tract (50, 113). Although NHP models of EVD recapitulate most features of human EVD, there are some differences between them. Notably, NHPs often exhibit a shorter disease course with uniform lethality, and both African green monkeys and common marmosets fail to develop a rash (45, 113, 115).

CLINICAL ILLNESS

Early Stages of Infection

Early stages of infection include asymptomatic incubation followed by the onset of nonspecific signs and symptoms associated with abnormal clinical laboratory findings. During incubation, patients are able to carry on with routine activities, including work and travel, but become quickly incapacitated upon illness onset. Clinical and laboratory findings during early stages of infection are discussed below.

Clinical findings. EVD should be suspected when a compatible clinical syndrome is associated with an epidemiologic risk factor, including contact with a suspected or confirmed case.

Initial nonspecific EVD symptoms (**Figure 5**) include malaise, fatigue, and muscle weakness and/or myalgia preceding or concurrent with fever onset (>38°C) (81). These early findings cannot, however, reliably differentiate EVD from other infections routinely observed in Africa, including malaria, leptospirosis, influenza and other respiratory viral infections, yellow fever, dengue and a number of other arboviruses, or enteric viral or bacterial infections, among others. Asymptomatic or mildly symptomatic EBOV infection has been anecdotally described (118, 119). Atypical presentation of EVD has been observed during pregnancy, with initial symptoms limited to mild abdominal pain and sparse contractions without fever (120).

EVD symptom onset typically precedes detection of viral RNA in the blood by quantitative reverse transcription polymerase chain reaction (qRT-PCR), which is believed to represent infectious virions. Viral kinetic patterns, as determined by qRT-PCR, differentiate between survivors and nonsurvivors (48, 121): Although the rate of viral RNA increase is similar in both groups,

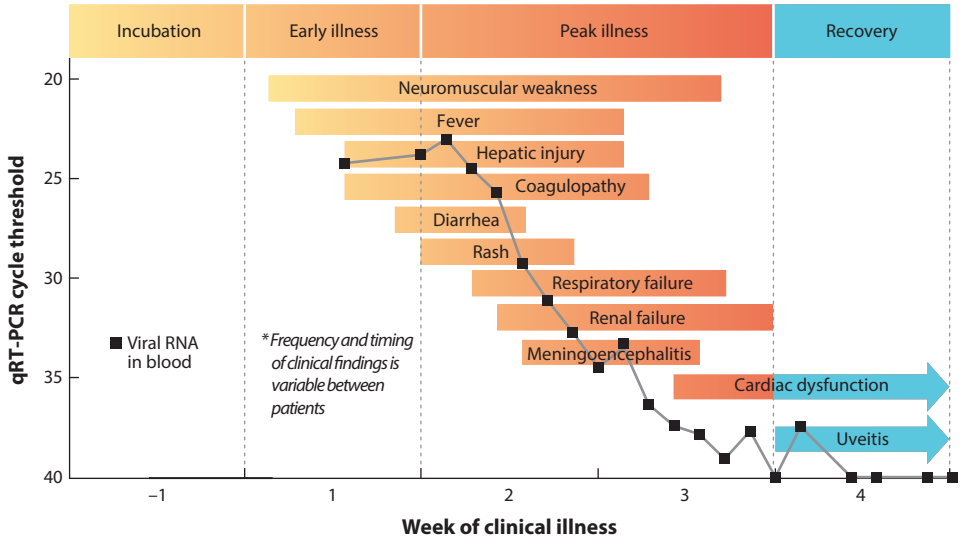

Figure 5

Clinical course of a typical case of severe Ebola virus disease. Detection of viral RNA in blood by quantitative reverse transcription polymerase chain reaction (qRT-PCR) (*line graph*), believed to represent viremia with infectious virions, occurs shortly after the onset of clinical illness. As disease progresses, sequential organ failure ensues despite declining viral RNA in blood. New or persistent clinical findings occur during recovery, following clearance of viral RNA in the blood. Figure adapted with permission from Reference 135.

those who go on to die typically have higher peak viral RNA levels associated with slower decline than do patients who survive (49, 122–124).

Progression to profound asthenia exacerbated by intractable nausea, vomiting, and profuse watery diarrhea usually occurs within the first week of illness (125). During this time, patients are highly infectious. Unless health care providers have adequate training and use personal protective equipment properly, nosocomial spread of EBOV may occur. Profuse watery diarrhea of up to 5–10 liters per day, similar in appearance to the rice water stools observed in cholera, begins around day 5 of illness and may last 7 or more days before tapering off (125, 126). Although mild inflammation has been seen histologically in the intestinal lamina propria (51), the large volume and watery consistency of stool suggest a primary secretory rather than inflammatory process, likely involving both the small and the large intestine (127). However, assays of intestinal inflammation, such as fecal leukocyte testing or stool lactoferrin or calprotectin assays, have not been reported, and viral or host-derived mediators of primarily secretory diarrhea have not yet been identified.

In the context of ongoing fluid and electrolyte losses, oral ulcers, dysphagia, odynophagia, and recurrent bouts of emesis may impair oral fluid intake. Without adequate fluid and electrolyte replacement, gastrointestinal fluid losses, exacerbated by losses from fever, tachypnea, and capillary endothelial leakage and intra- to extravascular volume shifts, may lead to severe dehydration and hypovolemic shock, contributing to organ failure and death (125).

Laboratory findings. As noted, viral or host mediators impair early immune responses and delay protective adaptive cellular and humoral responses. Early clinical laboratory findings (**Table 3**) include leukopenia and lymphopenia (128, 129). Total white blood cell counts frequently rebound and become elevated in conjunction with neutrophilia (129). Recovery of lymphocyte counts and high-level activation of EBOV antigen–specific and –nonspecific T- and B-cell responses have been observed in human survivors (130) and persist into convalescence.

Hemoglobin and hematocrit levels are elevated early in the course of EVD, prior to or concurrent with gastrointestinal fluid losses (128). Other factors that might contribute to hemoconcentration include evaporative fluid loss due to high ambient temperatures in tropical settings, diminished oral intake, and vascular leakage (129, 131). Anemia occurs later in illness, with average hemoglobin levels <11 mg/dl observed in patients treated in the United States and Europe. (In many African settings, anemia is common in otherwise healthy adults, a result of numerous factors including iron deficiency and helminth infections.) Anemia in EVD may be attributable to hemorrhage within tissues, bleeding from mucosal surfaces, or possibly hemolysis (51, 128). Clinically significant hemorrhage, manifested as hematemesis or hematochezia, occurs in only approximately 5% of cases, typically as a preterminal event (125, 129).

Table 3 Common laboratory findings in ebola virus disease

Timing	Laboratory finding
Early illness	■ Leukopenia, lymphopenia, and thrombocytopenia ■ Elevated hemoglobin and hematocrit ■ Elevated aspartate aminotransferase and alanine aminotransferase (ratio ≥3:1) ■ Elevated prothrombin time, activated partial thromboplastin time, and D-dimer
Peak illness	■ Leukocytosis, neutrophilia, and anemia ■ Hyponatremia, hypo- or hyperkalemia, hypomagnesemia, hypocalcemia, hypoalbuminemia, hypoglycemia ■ Elevated creatinine phosphokinase and amylase ■ Elevated blood urea nitrogen and creatinine ■ Elevated serum lactate and low serum bicarbonate
Recovery	■ Thrombocytosis

Abnormalities in serum electrolytes may progressively worsen in the absence of close monitoring and fluid and electrolyte replacement. Hyponatremia, attributable to electrolyte-rich enteric fluid losses and intravascular volume depletion, occurs in >30% of EVD cases (128). Replacement of severe diarrhea losses with an oral rehydration solution with reduced osmolality (e.g., more free water), rather than with balanced electrolyte solutions, may exacerbate hyponatremia. Hypokalemia and hypomagnesemia, resulting from gastrointestinal electrolyte losses, are also commonly observed during acute illness. Hyperkalemia, attributable to renal failure, is commonly observed in fatal cases (128, 129). Hypoalbuminemia develops early in the course of illness in most cases of EVD as a result of either urinary protein loss, extravasation into tissues, or possibly protein-losing gastroenteropathy, as documented in other acute viral infections (129, 132, 133). Hypocalcemia may occur in up to 75% of EVD cases, consistent with rates observed in patients who are critically ill with other diseases, where it is thought to be related to impaired parathyroid hormone (PTH) secretion, reduced calcitriol production, or end-organ resistance to PTH (129, 134).

Elevated serum transaminases are observed within the first week of EVD onset. Aspartate aminotransferase (AST) is more highly elevated than alanine aminotransferase (ALT), typically in a 3:1 or greater ratio, suggesting that some AST elevation may in part be attributable to extrahepatic sources including muscle insult (84, 128). Serum creatine phosphokinase levels are elevated in most EVD patients, consistent with myositis; levels >5,000 IU/L, suggestive of rhabdomyolysis, are observed in approximately one third of cases (84, 128). Peak AST levels >2,000 IU/L are more commonly observed in fatal cases (128). Following the rise in serum transaminases, mildly elevated serum total bilirubin levels (>1.5mg/dl) suggest that intrahepatic cholestasis may follow hepatocellular injury (129, 135).

DIC, manifested by elevations in prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimers, and thrombocytopenia, is observed within a week of EVD symptom onset (135). NHP data suggest EBOV triggers up-regulation of tissue factor expression on infected monocytes and macrophages (69). Circulating and resident tissue populations of these cells (e.g., Kupffer cells) likely contribute to early widespread initiation and propagation of the clotting cascade. Microvascular fibrin thrombi, deposited in multiple organs of NHPs, are implicated in the pathogenesis of central nervous system pathology in humans (135). Although thrombocytopenia is observed early in illness, platelet counts soon rebound and coagulation assays (PT, aPTT, D-dimers) may normalize as early as the second week of illness (126, 135, 136). Reactive thrombocytosis has been observed during recovery in up to one third of cases (129, 137).

Peak of Illness in the Second Week

The second week of EVD, characterized by stable or declining viral RNA in the blood and presumably decreasing viremia, is paradoxically associated with maximal organ injury leading to death in a subset of cases; those who survive this stage rapidly develop life-saving adaptive immune responses (49, 125, 138, 139). Sequential organ failure may occur despite meticulous fluid and electrolyte replacement and absent hypotension, apparently as a result of direct viral and indirect host-mediated organ injury (135). Hypoglycemia during EVD likely reflects depleted liver and muscle glycogen stores following high metabolic demand associated with severe illness.

Organ failure. Central nervous system (CNS) dysfunction is often observed in EVD. Life-threatening meningoencephalitis, characterized clinically by stiff neck, diminished consciousness, and such upper motor neuron signs as sustained clonus and hyperreflexia, is relatively common (125, 135). Meningoencephalitis has also been described shortly after apparent recovery and as a late complication (48, 140). Detection of live EBOV in the cerebrospinal fluid of a patient with meningitis months after initial clinical recovery from EVD suggests a direct viral contribution to

pathogenesis. It is also noteworthy that encephalitis has been histologically confirmed in two fatal human cases of MARV infection (141, 142) and in five of six EBOV-infected NHPs exhibiting delayed time to death following experimental therapy (143). Detection of viral antigen by IHC staining in glial nodules of these animals suggests that microglial cells may serve as a viral reservoir (143). Brain MRI findings during early convalescence in a patient with severe meningoencephalitis and focal neurological deficits suggested that microvascular occlusion contributed to tissue ischemia within the brain and spinal cord (135).

Hypo- or hyperactive delirium in EVD might result from electrolyte abnormalities, hypoglycemia, uremia, or possibly the hyperammonemia caused by severe liver damage. Neuromuscular weakness in EVD may be life threatening because it can lead to inability to care for oneself and can also contribute to respiratory failure (135). Other well-characterized neurologic findings include peripheral neuropathy, dysesthesia, hypothalamic dysfunction, seizures, and coma (81, 121, 125, 135, 144).

Acute kidney injury (AKI), which may progress to oliguric or anuric renal failure, is also frequently observed in EVD (128). EBOV infects renal tubular epithelial cells and glomerular endothelium and is detected in the interstitium. Autopsy findings in fatal human EVD confirm that infection results in acute tubular necrosis (51). Direct viral cytopathic effects, hypotension, and myoglobin-induced injury likely all contribute to renal failure. Renal failure has been shown to be reversible among survivors following short-term renal replacement therapy (145); renal recovery correlates with declining viral RNA in the blood (135). Urinary protein has not been quantified for EVD, although it would be useful in assessing the contribution of glomerulonephropathy to renal dysfunction. Fluid management may avert anuria even in cases of severe AKI (135).

Nonspecific signs and symptoms suggesting adrenal insufficiency in EVD include fatigue, nausea, vomiting, confusion, and electrolyte abnormalities (such as hyponatremia or hyperkalemia). In some patients, shock that is fluid-responsive may become unresponsive to fluids as the disease progresses; as in other critical illnesses where shock becomes fluid-refractory, high-dose vasopressor administration may be required to maintain blood pressure. In this setting, administration of stress-dose steroids for possible adrenal insufficiency should be considered.

Historically, respiratory failure was not thought to be a meaningful part of EVD pathogenesis because respiratory problems have not been prominently described in African settings where aggressive fluid replacement was uncommon, and because tachypnea in terminal stages of EVD was thought to be related to compensatory mechanisms of terminal shock (81). However, recent observations among patients cared for in Europe and the United States confirm that hypoxia and respiratory failure are common in EVD, affecting up to 30% of patients (129). Noncardiogenic pulmonary edema, pleural effusion, and hypoxia have been observed within the first week of illness and may potentially be worsened by aggressive fluid resuscitation for management of gastrointestinal fluid losses (131, 136).

EBOV-infected alveolar macrophages, seen by IHC in the lungs of human patients with fatal EVD, may contribute to inflammation and pulmonary vascular leakage (51). Severe inflammation of the airway epithelium (i.e., trachea, bronchi, bronchioles) or the alveoli has not been described in fatal cases (51), and progressive and refractive hypoxia, as occurs in severe respiratory viral infection (e.g., influenza), does not appear to be a prominent feature of respiratory failure in EVD (129). Ventilation/perfusion mismatch due to pulmonary vascular thrombi, observed in NHPs, may further contribute to hypoxia (143). Profound muscle weakness predisposes infected individuals to aspiration pneumonia, and ineffective dead space ventilation limits alveolar gas exchange.

Other organ dysfunction. EBOV induces dysfunction of multiple other organ systems. Ocular disturbances including conjunctival injection, eye pain, and blurred vision have been described during acute illness (86). Corneal involvement has been confirmed microscopically in NHPs, with

the virus infecting epithelial, stromal, and endothelial cell layers (143). Clinical findings suggest that uveitis complicates acute human EVD and is also a late complication (85).

A diffuse, nonpruritic, erythematous maculopapular rash, often less apparent in dark-skinned individuals, may appear during the first week of illness. During the second week the rash becomes confluent, manifesting as diffuse erythroderma, and then resolves with desquamation (146). Human data confirm that multiple cell types within the skin, including fibroblasts, dendritic cells, endothelium, and epithelium of sweat and sebaceous glands, may be infected by EBOV (51).

The multiple cardiac findings observed in EVD are likely due to direct and indirect viral effects that result in structural and functional cardiac abnormalities. Pericarditis with serous pericardial fluid was found in a fatal case of EVD in which the patient had retrosternal pain during the course of illness (81); pericardial fluid has also been detected by cardiac ultrasound during acute illness in a survivor (136). Mild myocardial dysfunction has been documented by echocardiogram during acute illness (131), and cardiac MRI has detected myocardial edema or inflammation with progression to fibrosis, consistent with myocarditis, during recovery (147). The clinical appearance of myocarditis due to other viruses (e.g., enteroviruses) may range from mild infection to progressive dilated cardiomyopathy, cardiogenic shock, and fatal arrhythmias. Although myocarditis leading to severe myocardial dysfunction and predisposition to fatal arrhythmias has been suspected in EVD, there are few data to support this possibility.

High fever early in the course of EVD has been associated with pulse–temperature dissociation (relative bradycardia) (146). Arrhythmias or electrocardiographic changes were documented in 41% of patients treated in Europe or the United States during 2014–2015 (129). In one of these patients, QT interval prolongation greater than 600 milliseconds occurred despite normal serum electrolytes and absent pharmacologic agents known to produce QT prolongation. Clinical observations from West Africa of unexplained sudden death in patients who were clinically improving are also consistent with fatal arrhythmias (125). Risk factors that predispose infected individuals to fatal arrhythmias, in particular to torsade de pointes (148), may include underlying structural and functional cardiac defects; combinations of abnormal serum magnesium, potassium, and calcium levels; and administration of drugs that prolong QT intervals, such as quinolone antibiotics, haloperidol, and certain antiemetics and antimalarial agents.

Diffuse abdominal pain and elevated serum amylase levels suggest that pancreatitis may also complicate EVD. Although abdominal pain is a nonspecific finding, and elevated amylase might derive from salivary or other sources, the possibility of pancreatitis is supported by detection of EBOV in the endocrine and exocrine pancreas of infected NHPs (143). EBOV is also detectable in the NHP thyroid following infection (143), suggesting the need for longitudinal evaluation of thyroid function in EVD survivors.

Concurrent infections. Malaria coinfection was detected in approximately 15% of EVD patients in a large treatment center in Liberia during 2014–2015, supporting the need to diagnose and empirically treat malaria in EVD, especially during seasonal periods of elevated malaria incidence (149). Gram-negative bacterial pneumonia and gram-negative sepsis have complicated individual cases of EVD managed in Europe and the United States (135, 136). The prevalence of bacterial coinfection in EVD has not, however, been determined. Despite inadequate data, empiric antimalarial and antibiotic use targeting enteric gram-negative organisms was common in ETUs during the 2013–2015 West Africa epidemic (125), as had been the case in some earlier outbreaks. Whether translocation of gut bacterial flora into the bloodstream meaningfully contributes to EBOV pathogenesis remains an important outstanding question.

Pregnancy. EVD results in high maternal and perinatal mortality, with most pregnancies ending in spontaneous miscarriage, stillbirth, or early neonatal death (150). EBOV crosses the placenta

to infect the fetus and the amniotic fluid. The virus disappears from the maternal circulation of survivors during later gestation but viral RNA levels may remain detectable in fetal circulation for a month or longer (151). Products of conception, including the placenta, amniotic fluid, and newborn infant of a mother who has recovered from EVD, must therefore be considered infectious.

Recovery and Convalescence

Resolution of viremia allows for a transition from tissue injury to repair. Clinical manifestations of disease, however, persist late into convalescence. As discussed below, persistence of live virus in select immune-privileged sites clearly contributes to some of the late sequelae of infection, whereas in other situations the causes of sequelae are less well defined.

Clinical findings. EVD survivors often experience late-onset sequelae. One EVD survivor developed clinical meningitis with isolation of live virus from the cerebrospinal fluid 9 months after initial recovery (152); another patient had uveitis with isolation of live virus from the aqueous humor 98 days following initial symptom onset (85). Uveitis affects approximately 20% of EVD survivors; visual loss and blurred vision affect approximately <5% and 40%, respectively (153). Other ocular symptoms include light sensitivity, tearing, pain, dryness, and burning. Nonocular findings in EVD survivors include fatigue, cognitive difficulties, muscle pain and weakness, arthralgia, tinnitus, and hearing loss that may persist for a year or longer (153, 154).

Viral persistence. The factors that trigger late manifestations of disease associated with the persistence of infectious EBOV virions in immune-protected sites, including the eye, CNS, and testis, are not fully understood. Studying this problem is complicated by the fact that most available data rely on viral RNA detection, which may or may not represent infectious virions capable of sustaining infection within organs. EBOV persists in the semen of some male survivors, increasing risk of sexual transmission from male survivors to their partners. Live virus has been isolated from the semen of a survivor 82 days following initial recovery (88); sexual transmission from a male survivor to his female partner 179 days after illness onset was supported by viral genome sequencing (155), and viral RNA has been detected in semen 531 days after onset of disease (152). Detection of viral RNA by qRT-PCR in tissues and body fluids often extends beyond the interval when live virus can be isolated. Ebola RNA has been detected in the urine of a survivor 9 days after disappearance of the virus from the blood (136), potentially due to sloughing of dead and dying renal tubular epithelial cells in the recovering urinary tract. These data are consistent with NHP experimental data that show live EBOV persistence in dead and dying tissues, with retention of infectivity up to 7 days after death (156). In addition to the results described above, the longest intervals between EVD onset and documentation of EBOV RNA in fluids and tissues are 40 days in sweat (136), 33 days in vaginal swabs (88), 30 days in urine (136), 29 days in rectal swabs (88), 26 days in breast milk (157), 25 days in stool (158), 8 days in saliva (159), and 6 days in tears (159). Implications for prolonged viral persistence and for viral transmission are unclear.

Treatment

Multiple antiviral compounds have shown therapeutic promise in in vitro and animal studies. Several of these were administered to EVD patients or to persons undergoing prospective clinical trial evaluation during the West African epidemic. To date, however, no EBOV-specific therapy has been proven efficacious, nor has any therapy achieved regulatory approval for use in humans. Consequently, supportive care remains the mainstay of treatment.

Targeted therapies. Favipiravir is a nucleoside analog that inhibits replication of multiple RNA viruses (160) and was shown to reduce mortality in EBOV-infected mice (161, 162). However, in a nonrandomized study conducted in Guinée (referred to as Guinea in many English-speaking countries) during 2013–2015, no reduction in viral load or mortality was observed among 99 EVD patients treated with favipiravir when compared to untreated historical control subjects (163). Convalescent plasma has been administered to individuals and to small cohorts of EVD patients for decades (164). In the largest study to date, conducted at a single center in Guinea during 2015, no difference in mortality was observed among 84 EVD patients who received two doses (200 and 250 mL) of ABO-matched convalescent EBOV plasma compared with 418 untreated historical control subjects (165). ZMapp, a cocktail of thee monoclonal antibodies (MAbs), has been shown to prevent the death of EBOV-infected macaques following onset of fever and viremia (166). Results of a randomized clinical trial of ZMapp therapy versus supportive care have not yet been published. Although uncontrolled clinical reports of possible ZMapp efficacy have raised hopes, it is not clear that ongoing studies, which have enrolled limited numbers of patients, some in late stages of disease, are of sufficient power to establish efficacy.

Multiple other compounds have been evaluated in preclinical studies for the treatment of EVD but have not been studied for safety and efficacy in humans. For example, compounds that interfere with viral messenger RNA synthesis, including TKM-Ebola (167, 168) and antisense oligonucleotides (169), or those that inhibit viral RNA polymerase function, including BCX4430 (170) and GS-5734 (171), have been associated with reduced EBOV mortality in animal models but have not been evaluated in controlled clinical studies. Similarly, therapy targeting disordered coagulation using recombinant nematode anticoagulant protein C2 (172) or recombinant activated protein C (173) has improved survival in macaques but has not yet advanced to human trials.

Supportive care. Supportive therapy in West Africa, Europe, and the United States has been associated with a dramatic reduction in case–fatality ratio compared with historical norms. Of the 27 patients treated in Europe and the United States, some of whom were admitted in late stages of illness, five died, an 18.5% case–fatality ratio compared to the universally higher case–fatality ratios observed in West Africa in 2013–2015, in most earlier epidemics, and in the 1967 Marburg outbreak as well. Some of the patients cared for in Western nations, however, also received experimental therapies such as antibodies, small molecules, or antivirals, which might have contributed favorably to their chances of survival.

To guide timely interventions, optimal intensive supportive therapy for EVD requires near-continuous bedside care to closely monitor vital signs, fluid and electrolyte status, and clinical complications. Noninvasive monitoring of blood pressure, cardiac telemetry (electrocardiogram), and oxygen saturation, as well as the use of indwelling venous catheters for serial sampling of blood to determine serum electrolyte levels and organ function and to administer intravenous therapies, greatly facilitate care. Use of bedside ultrasound to assess cardiac function and intravascular volume status is also of great value. Analyses of serum chemistries, lactate level, and blood gases provide a basic complement of point-of-care laboratory testing. Assessment of complete blood cell counts with differential and coagulation studies also provide valuable data. Pharmacotherapy includes the use of antiemetics, antidiarrheal medication, analgesics, sedatives, antibiotics, and antimalarial medications, among others. Selection of drugs should take into account sedative effects, organ toxicity (e.g., renal and hepatic), and association with cardiac arrhythmias. Nutrition may be provided by enteral or parenteral routes as tolerated.

Hypoxia may be managed with administration of supplemental oxygen, but respiratory failure requires mechanical ventilation. Invasive mechanical ventilation may also be needed for airway protection when consciousness is severely impaired due to sedation or encephalopathy.

Prevention of intravascular volume depletion and avoidance of nephrotoxic agents may limit acute kidney injury, and renal failure may be managed with renal replacement therapy (145). Adequate supportive care during sequential multiple organ failure allows time for return of EBOV-specific humoral and cellular immune responses, viral clearance, and near-complete recovery from organ dysfunction. Resolution of disability and sequelae from EVD, however, may require a year or longer. The question remains how to properly implement and execute appropriate supportive care in infrastructure-poor environments such as many places in equatorial Africa.

PREVENTION OF HUMAN EBOLA VIRUS DISEASE

Although ebolaviruses emerge across an enormous belt of tropical Africa (**Figure 2**), areas of putative high risk have been identified (31), allowing for continual vigilance, enhanced surveillance, and education of the public. Because EVD outbreaks have frequently been widely exported, rapid exchange of information between countries and between locales within countries allows the identification of importations, facilitating rapid control efforts to prevent spread, as was the case in Nigeria in 2014 (174). Key to controlling ebolavirus epidemics are prompt identification, diagnosis, and safe transport of patients to hospitals; isolation and monitoring of their contacts; epidemiologic assessment; and, when possible (under vaccine clinical trial situations), vaccination of health care workers, of those involved in patient transport, burial, and cleanup of infectious materials, and of others who are at high exposure risk, as well as vaccination of primary and secondary contacts (ring vaccination). Safe burial practices emphasizing a lack of direct contact with the dead are critical (40), as unsafe burial practices appear to be a major mechanism of ebolavirus spread through African communities.

No ebolavirus vaccine has yet been licensed (as of December 2016), but 11 different EBOV vaccines are or have recently been undergoing clinical study (175). Four of these are now in Phase III clinical testing; one has shown efficacy under the circumstances of a specific experimental study of ring vaccination. A recombinant replication-competent vesicular stomatitis virus expressing the EBOV GP (referred to as rVSV-ZEBOV), studied in Guinea during the epidemic in 2015, showed 100% efficacy (CI 74.7–100%) in 7,651 subjects enrolled in a ring vaccination trial in which some subjects were vaccinated on day 0 and others on day 21 (176). This vaccine is also currently under investigation in the PREVAIL and STRIVE vaccine trials in Liberia and Sierra Leone, respectively (177, 178).

Other EBOV vaccines in Phase III testing include a vectored nonreplication-competent chimpanzee adenovirus type 3 vaccine (ChAd3-EBO) and related EBOV vaccines based on chimpanzee adenovirus types 5 or 26, each of which expresses ebolavirus GP. A major disadvantage of these vaccines is the requirement for vaccine boosting at a yet to be determined interval (probably less than 28 days), but the vaccines have a strong safety profile, and one formulation immunizes against both EBOV and SUDV. A monovalent EBOV version of this vaccine is currently undergoing testing as an arm of the Phase III PREVAIL trial in Liberia. A fourth vaccine, based on the replication-competent vaccinia virus known as MVA (modified vaccinia Ankara), expresses the GPs of EBOV, SUDV, and MARV, as well as the nucleoprotein of TAFV. This vaccine, known as MVA-BN-Filo, was quickly developed for study in the West African epidemic either as a stand-alone vaccine or as a prime or boost in prime-boost sequential vaccination in conjunction with a chimpanzee adenovirus vaccine.

One potential challenge to ebolavirus vaccination is the essential impossibility of predicting where and when ebolaviruses will emerge. Unless a vaccine is used in universal vaccination programs of very large healthy populations, something not currently envisioned, it will be necessary to quickly deploy stockpiled vaccine to chase epidemics as they emerge and spread unpredictably,

making it likely that many people will not have been vaccinated by the time they are first exposed to the virus. Nevertheless, vaccinating persons at high risk, such as health care workers, those involved in burials, and contacts of cases, would play a clear role in prevention of disease. Another problem is that most current vaccines only immunize against EBOV and would not be useful in outbreaks caused by other ebolavirus species.

SUMMARY

Accumulating evidence about the pathogenetic mechanisms of severe EVD provides a basis for significantly decreasing EVD mortality though early detection and early institution of supportive care and specific treatment. It has become clear that EVD is not invariably fatal and that optimal treatment, instituted early on, can result in higher rates of survival with relatively lower rates of severe sequelae. Early intervention is critical: Once cascading events have damaged multiple organs, the chances of supporting life until viral replication ceases, and natural tissue repair returns, are greatly reduced.

The natural history and pathogenesis of EVD includes a moderately long incubation period during which time the virus, exhibiting tropism for many different cell types, spreads rapidly to infect the entire reticuloendothelial (monocyte/macrophage) system in most organs of the body, quickly spreads to the surrounding parenchymal tissues within these organs, is further disseminated via the bloodstream and, after the host overcomes early viral immune suppression, induces a robust immune response, which can clear infection from all but immunologically privileged sites but which may also, paradoxically, contribute to disease severity. The ability of cells and organs to ultimately recover from EVD suggests that survival can be expected for many if not most patients if they can be identified and treated early in the illness course and medically supported throughout the acute stages (179). (The extent of full recovery, however, remains an open question, as the incidence of long-term sequelae is currently unknown.) In Western nations, such care is available in specialized ICU settings. In remote African settings, such care is currently unavailable. An important focus of future research will be how to optimize EVD care and reduce mortality in the resource-poor settings where Ebola continues to emerge.

Comprehensive prevention includes anticipation of outbreaks in geographic areas of risk (31). Adopting public health preventive measures such as intensive community-wide surveillance and immediate notification of suspected new cases; tracing, isolating, monitoring, and testing of EVD contacts; immediate placement of contacts under health monitoring to prevent tertiary spread; and, should such subjects develop EVD, providing early optimal supportive care and specific treatment. When possible, vaccination of all health-care and high-risk workers in the affected area and of all patient contacts, as well as their secondary contacts (ring vaccination), should be carried out; when feasible, community-wide vaccination should be carried out as well. Community education and involvement, leading to a trusting relationship with medical and public health officials, is of great importance.

EBOV prevention infrastructures of the type described above were set up over much of West Africa in 2014–2015 but may decline over time and must be quickly established de novo when ebolaviruses emerge elsewhere. Because ebolaviruses usually emerge in remote areas, rapid establishment of prevention and treatment infrastructures will typically require importation of outside equipment, supplies, and experts in medical care, public health, and viral diagnosis. These infrastructures will also require logistical support, which may include transportation, electrical generation, water purification, and construction of treatment units. At present, there is no well-organized global entity to quickly provide this sort of rapid comprehensive epidemic support, but these challenges are currently under discussion at many national and international levels. In

planning the responses to future African ebolavirus outbreaks, emphasis must therefore be placed on rapidity of effort, early case detection with immediate institution of care and treatment, and optimization of supportive and specific care using all available and deployable resources, with the aim of establishing treatment environments and capacities that approach, to the greatest extent possible, those available in Western ICU settings.

SUMMARY POINTS

- For the past 40 years, EVD has continually reemerged from unknown reservoirs in numerous locales across the equatorial belt of Africa and is likely to continue doing so for the foreseeable future.
- Although incompletely characterized, the pathogenesis of severe EVD reflects damaging systemic viral infection of multiple cell types in multiple organs, associated with both early immune evasion and subsequent immune damage.
- Even in the face of life-threatening EVD, tissue repair and recovery begin promptly; early, aggressive supportive care may substantially reduce mortality.
- During the recent West African EBOV epidemic, promising vaccines, antiviral compounds, and therapeutic monoclonal antibodies were tested, and one vaccine was demonstrated to be efficacious.
- Until such time as efficacious vaccines and drugs can be fully tested, produced, and stockpiled, control of Ebola will depend upon aggressive surveillance, public health responses, and stronger partnerships between medical, public health, social, and cultural players in involved communities.

FUTURE ISSUES

- An important challenge is optimizing EVD care in settings with limited resources, where the disease typically reemerges.
- Better understanding of ebolavirus disease mechanisms is needed to guide development of drugs, vaccines, and treatment strategies.
- Testing vaccines and drugs will remain challenging, as will responding to future epidemics with public health and medical capacity, because ebolavirus reemergences occur at unpredictable times and places, and many such emergences may evolve rapidly.

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