Chapter 23

FILOVIRUSES

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INTRODUCTION

Human viral hemorrhagic fevers are typically caused by members of the four families: (1) *Arenaviridae* (several mammarenaviruses), (2) *Bunyaviridae* (several hantaviruses, nairoviruses, phleboviruses), (3) *Filoviridae* (certain ebolaviruses, marburgviruses), and (4) *Flaviviridae* (several flaviviruses *sensu stricto*).[1] The viruses of these four families are distinct in their molecular biology, reservoir host spectrum, and transmission route. However, the human diseases these viruses cause are clinically and pathologically similar, and all of the diseases are associated with significant lethalities (case fatality rates).[1] Among these viruses, filoviruses are arguably the most notorious as they are associated with the highest lethality and receive the widest attention in the media.[2] Importantly, filoviruses were included in the Soviet biological weapons research program.[3] The actual achievements of this program are still under debate, but through their inclusion, filoviruses gained military significance. This chapter provides an overview of the diversity, epidemiology, and molecular biology of filoviruses; summarizes the clinical presentation and pathology of the human diseases they cause; and reviews current developments in prophylactics and antivirals for the prevention and treatment of infections.

NOMENCLATURE

Filovirus Taxonomy

According to the International Committee on Taxonomy of Viruses, the family *Filoviridae* is one of seven families included in the order *Mononegavirales*.[4,5] The eight members of the family *Filoviridae*, referred to as filoviruses, are assigned to the three genera—*Cuevavirus*, *Ebolavirus*, and *Marburgvirus*—based on the evolutionary/phylogenetic relationship of their coding-complete genome sequences and differences in biological properties of their virions (Figure 23-1).[4–6] The members of the three genera, referred to as cuevaviruses, ebolaviruses, and marburgviruses, respectively, also differ in their geographic distribution, virion antigenicity, and overall genome organization. The International Committee on Taxonomy of Viruses *Filoviridae* Study Group recognizes one cuevavirus, Lloviu virus (LLOV); five ebolaviruses, Bundibugyo virus (BDBV), Ebola virus (EBOV), Reston virus (RESTV), Sudan virus (SUDV), and Taï Forest virus (TAFV); and two marburgviruses, Marburg virus (MARV) and Ravn virus (RAVV) (Table 23-1, Figure 23-1).[6,7] The different isolates of each filovirus are grouped into variants, which typically correspond to viruses circulating in particular human disease outbreaks.[8] For instance, several EBOV isolates were obtained during a disease outbreak in 1976 in Zaire (Democratic Republic of Congo).[9] These isolates are more closely related to each other than to several EBOV isolates obtained during a disease outbreak in Guinea in 2014. These two groups of viruses are therefore assigned different variant names (in this case, Yambuku and Makona, respectively).[10,11] The term "strain" is reserved for nonnatural, laboratory-animal-adapted or certain cDNA-derived filoviruses.[12,13]

Filovirus Disease Nomenclature

With the exception of LLOV and RESTV, all other filoviruses are associated with severe human illness. In the *International Statistical Classification of Diseases and Related Health Problems* by the World Health Organization, human disease names are standardized internationally. In its most current version, *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10)*, two filovirus diseases are distinguished: (1) Marburg virus disease (MVD; colloquially often referred to as Marburg hemorrhagic fever), which is caused by MARV or RAVV; and (2) Ebola virus disease (EVD; colloquially often referred to as Ebola hemorrhagic fever), which is caused by BDBV, EBOV, SUDV, or TAFV (Table 23-2).[14]

Filovirus Categorization

All filoviruses are considered World Health Organization Risk Group 4 infective microorganisms. Therefore, any research involving replicative forms of the viruses must be performed in maximum containment facilities.[15] In the United States, such facilities are designated as (animal) biosafety level 4 laboratories. Given the associated high lethality with infections and the absence of licensed medical countermeasures (MCMs), filoviruses are considered high-consequence pathogens and are therefore categorized as Centers for Disease Control and Prevention (CDC) Bioterrorism Category A Agents[16] and National Institute of Allergy and Infectious Diseases Category A Pathogens.[17] Categorized as Tier 1 Select Agents, access to replicative forms of filoviruses is highly restricted by law,[16] and their export is tightly controlled.[18]

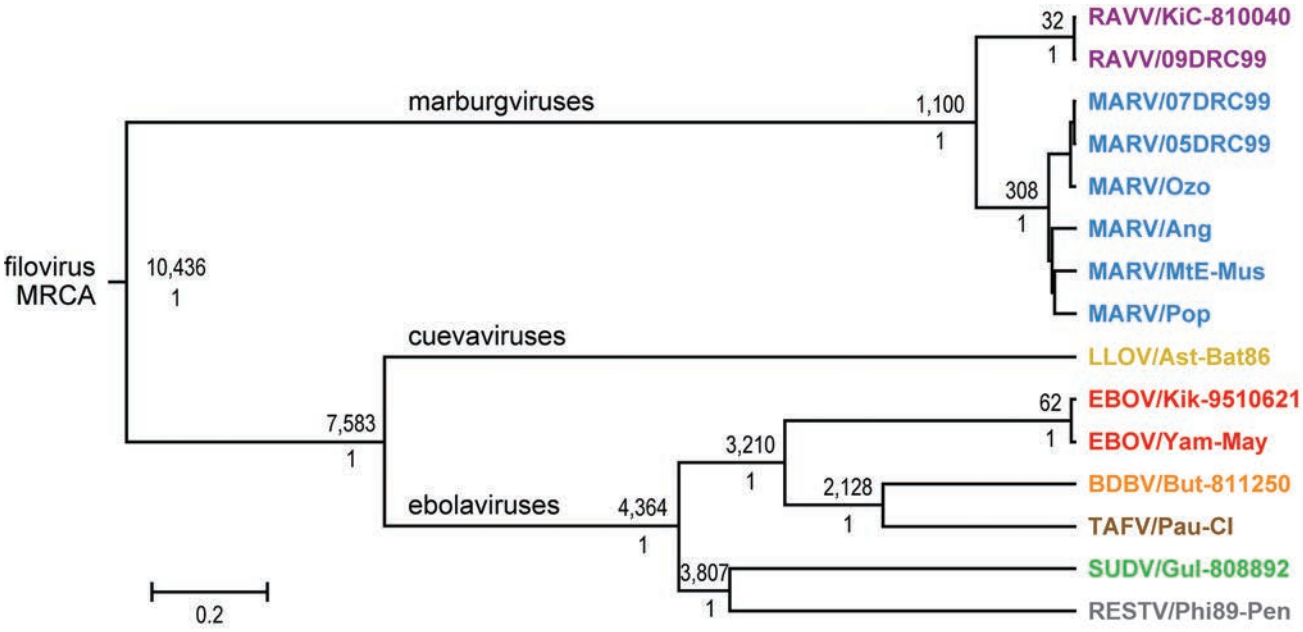

Figure 23-1. Phylogenetic relationships of members of the family *Filoviridae*. Bayesian coalescent analysis of representative cuevaviruses, marburgviruses, and ebolaviruses. Shown is the maximum clade credibility tree with the most recent common ancestor number at each node. Posterior probability values are shown beneath the most recent common ancestor estimates in years. The scale is in substitutions/site (based on data published by Serena Carroll/CDC). Appended to the virus abbreviation via a "/" is the variant abbreviation (eg, KiC, MtE, Ast, Yam, But, Pau, Gul, Phi) connected by a hyphen to the isolate designation (not all variant names are yet standardized, see data sources 3 and 4). MRCA: most recent common ancestor
Colors assigned to viruses in this table will be used in follow-up tables and figures: RAVV: *purple*; MARV: *blue*; LLOV: *yellow*; EBOV: *red*; BDBV: *orange*; TAFV: *brown*; SUDV: *green*; and RESTV: *gray*.
Data sources: (1) Carroll SA, Towner JS, Sealy TK, et al. Molecular evolution of viruses of the family *Filoviridae* based on 97 whole-genome sequences. *J Virol*. 2013;87:2608–2616. (2) Peterson AT, Bauer JT, Mills JN. Ecologic and geographic distribution of filovirus disease. *Emerg Infect Dis*. 2004;10:40–47. (3) Kuhn JH, Bao Y, Bavari S, et al. Virus nomenclature below the species level: a standardized nomenclature for natural variants of viruses assigned to the family *Filoviridae*. *Arch Virol*. 2013;158:301–311. (4) Kuhn JH, Andersen KG, Bào Y, et al. Filovirus RefSeq entries: evaluation and selection of filovirus type variants, type sequences, and names. *Viruses*. 2014;6:3663–3682.

Other countries differ from the United States and each other in the extent of implemented regulations or laws in regard to filovirus access and distribution. However, a worldwide general consensus exists on the overall threat associated with filoviruses and the need for proper containment.

MOLECULAR BIOLOGY

Filovirion Structure

Filoviruses are viruses that produce virions with filamentous morphology. Filovirions, which are enveloped particles that are greater than 800 nm long and approximately 90 nm in diameter, are covered with spike protrusions of approximately 10 nm long. The particles are flexible and appear pleomorphic, assuming shapes that are reminiscent of spaghetti (Figure 23-2), but they can also be branched or circularized.[19–25]

Filovirus Genomes and Proteins

Complete filovirions contain one or more genome copies.[23] Each genome is a monopartite, approximately 19 kb long, linear, uncapped, and polyadenylated single-stranded RNA of negative polarity that has 3′ and 5′ complementary termini. All filoviruses contain genomes with the same linear arrangement of six (LLOV) to seven genes (all other filoviruses) in the order 3′-NP-VP35-VP40-GP-VP30-VP24-L-5′.[26,27] However, the various filovirus genomes differ from each

TABLE 23-1

FILOVIRUS CLASSIFICATION AND NOMENCLATURE

2010 to Present	Outdated Virus Names and Abbreviations
Order *Mononegavirales*	
Family *Filoviridae*	
Genus *Marburgvirus*	
Species *Marburg marburgvirus*	
Virus 1: **Marburg virus (MARV)**	Marburg virus (MBGV), Lake Victoria marburgvirus
Virus 2: **Ravn virus (RAVV)**	Marburg virus (MBGV), Lake Victoria marburgvirus
Genus *Ebolavirus*	
Species *Bundibugyo ebolavirus*	
Virus: **Bundibugyo virus (BDBV)**	Bundibugyo virus (BEBOV)
Species *Reston ebolavirus*	
Virus: **Reston virus (RESTV)**	Reston ebolavirus (REBOV)
Species *Sudan ebolavirus*	
Virus: **Sudan virus (SUDV)**	Sudan ebolavirus (SEBOV)
Species *Taï Forest ebolavirus*	
Virus: **Taï Forest virus (TAFV)**	Côte d'Ivoire ebolavirus (CIEBOV), Ivory Coast ebolavirus (ICEBOV)
Species *Zaire ebolavirus*	
Virus: **Ebola virus (EBOV)**	Zaire ebolavirus (ZEBOV)
Genus *Cuevavirus*	
Species *Lloviu cuevavirus*	
Virus: **Lloviu virus (LLOV)**	

In taxonomy, taxa (orders, families, genera, and species; recognizable by italicization) are considered concepts of the mind that do not have properties. Taxa are represented by physical members, the viruses (names in color). Only virus names are to be abbreviated in technical writing. See Figure 23-1 for color explanations.
Data sources: (1) Kuhn JH, Becker S, Ebihara H, et al. Family *Filoviridae*. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, eds. *Virus Taxonomy—Ninth Report of the International Committee on Taxonomy of Viruses*. London, United Kingdom: Elsevier/Academic Press; 2011:665–671. (2) Kuhn JH, Becker S, Ebihara H, et al. Proposal for a revised taxonomy of the family *Filoviridae*: classification, names of taxa and viruses, and virus abbreviations. *Arch Virol*. 2010;155:2083–2103. (3) Bukreyev AA, Chandran K, Dolnik O, et al. Discussions and decisions of the 2012–2014 International Committee on Taxonomy of Viruses (ICTV) *Filoviridae* Study Group, January 2012–June 2013. *Arch Virol*. 2014;159:821–830.

other in sequence and in the number of gene overlaps, intergenic regions, and the proteins expressed from the GP gene (Figure 23-3).[26–29] The seven filovirus genes, NP, VP35, VP40, GP, VP30, VP24, and L, encode at least seven structural proteins, respectively: nucleoprotein (NP), polymerase cofactor (VP35), matrix protein (VP40), glycoprotein ($GP_{1,2}$), transcriptional activator (VP30), secondary matrix protein (VP24), and RNA-dependent RNA polymerase (L).[23,26,27,30,31] In the case of cuevaviruses and ebolaviruses, three nonstructural proteins are encoded: secreted glycoprotein (sGP), secondary secreted glycoprotein (ssGP), and Δ-peptide (Table 23-3).[32–34] $GP_{1,2}$ is also converted into a nonstructural secreted product ($GP_{1,2\Delta}$) by tumor necrosis factor α-converting enzyme.[35] Under certain circumstances, sGP may become a structural component of ebolavirions.[36] Five of the main structural proteins—NP, VP35, VP40, $GP_{1,2}$, and L—are clearly functional analogs of the standard set of mononegaviral core proteins (N, P, M, G, and L, respectively).[37]

Filovirus Lifecycle

Filovirions bind to attachment factors on the host cell surface[38] via $GP_{1,2}$, a type 1 transmembrane and class I fusion protein,[39,40] which determines filovirus host and cell tropism.[41] After cell surface binding, filovirions enter the cell through endocytosis.[42–44] In the endolysosome, after a proteolytic cleavage that reveals the receptor-binding site, $GP_{1,2}$ engages Niemann-Pick C1 protein,[45,46] which triggers a complex $GP_{1,2}$ refolding process ensuing in fusion of the endolysosomal membrane and the virion envelope.[47] The result of this fusion is the release of the filovirus ribonucleoprotein (RNP) complex into the cytosol, where filovirus replication occurs.

At the core of the RNP complex is a helical polymer of NPs that serves as a scaffold for the filovirus genome and VP40 and VP24, which wrap around the helix.[21–23] The functional polymerase complex, which is part of the RNP, consists of filoviral L, VP35, and filovirus-unique VP30, and is bound to the filoviral genome.[48,49]

TABLE 23-2

FILOVIRUS DISEASE CLASSIFICATION AND NOMENCLATURE

ICD-10 (1990–Present)	Informal Designations
A98.3: Marburg virus disease (MVD) Caused by: **Marburg virus (MARV)** **Ravn virus (RAVV)**	Marburg hemorrhagic fever (MHF)
A98.4: Ebola virus disease (EVD) Caused by: **Bundibugyo virus (BDBV)** **Ebola virus (EBOV)** **Sudan virus (SUDV)** **Taï Forest virus (TAFV)**	Ebola hemorrhagic fever (EHF)

See Figure 23-1 for color explanations.
ICD-10: *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision*
Data source: World Health Organization. *International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10)*. http://apps.who.int/classifications/icd10/browse/2015/en. Accessed September 22, 2015.

Upon release into the cytosol, these complexes move along the filoviral genome in the infected cell and transcribe the six/seven filoviral genes into polyadenylated typically monocistronic mRNAs that are then translated, or replicate the entire NP-encapsidated genome into encapsidated antigenomes and back into encapsidated progeny genomes.[50,51] VP40 and VP24, which regulate these two processes,[52] also regulate virion morphogenesis in the cell by recruiting newly formed RNPs and play major parts in the filovirion budding process from host cell membranes. Filoviruses bud through endosomal multivesicular bodies followed by exocytic release or via direct budding through the plasma membrane at membrane/lipid rafts.[53–57] $GP_{1,2}$ is expressed and proteolytically cleaved into its two subunits (GP_1 and GP_2) during transport through the secretory pathway of the infected cell, and trimers of GP_1-GP_2 heterodimers are transported to and inserted into host cell membranes.[58,59] Budding filovirions, which acquire their envelopes from the host cell membrane during egress, therefore also acquire the inserted $GP_{1,2}$, which are the spikes seen on the filovirion surface in electron microscopy sections.

Geographic Distribution

The still undefined geographic distribution of filoviruses in nature is deduced from natural host reservoir studies, epizootiology, epidemiology, serological surveys, and ecological niche modeling.[2]

Natural Reservoirs of Filoviruses

Although numerous studies were performed,[2] the natural host(s) for BDBV, EBOV, LLOV, RESTV, SUDV, and TAFV remain elusive. MARV and RAVV are the only filoviruses for which at least one natural

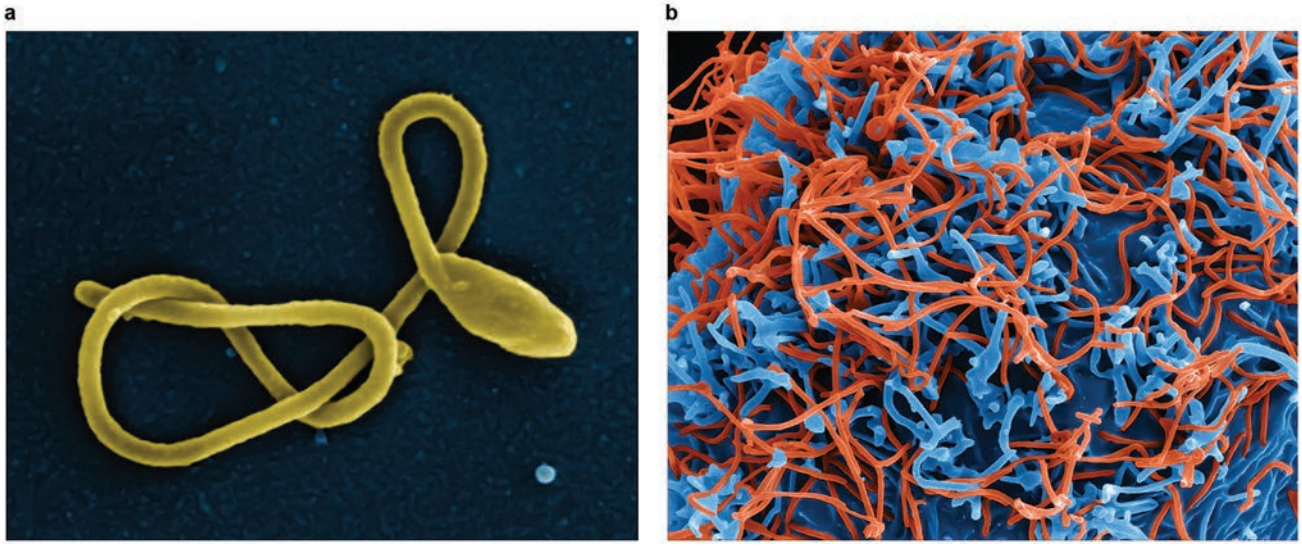

Figure 23-2. Filovirion structure. (**a**) Colorized scanning electron micrograph of a single filamentous Ebola virion (original magnification × 100,000). (**b**) Colorized scanning electron micrograph of filamentous Ebola virions (*red*) budding from a chronically infected grivet (Vero E6) cell (*blue*) (original magnification × 35,000).
Photographs: Courtesy of John Bernbaum and Jiro Wada, Integrated Research Facility at Fort Detrick, Maryland.

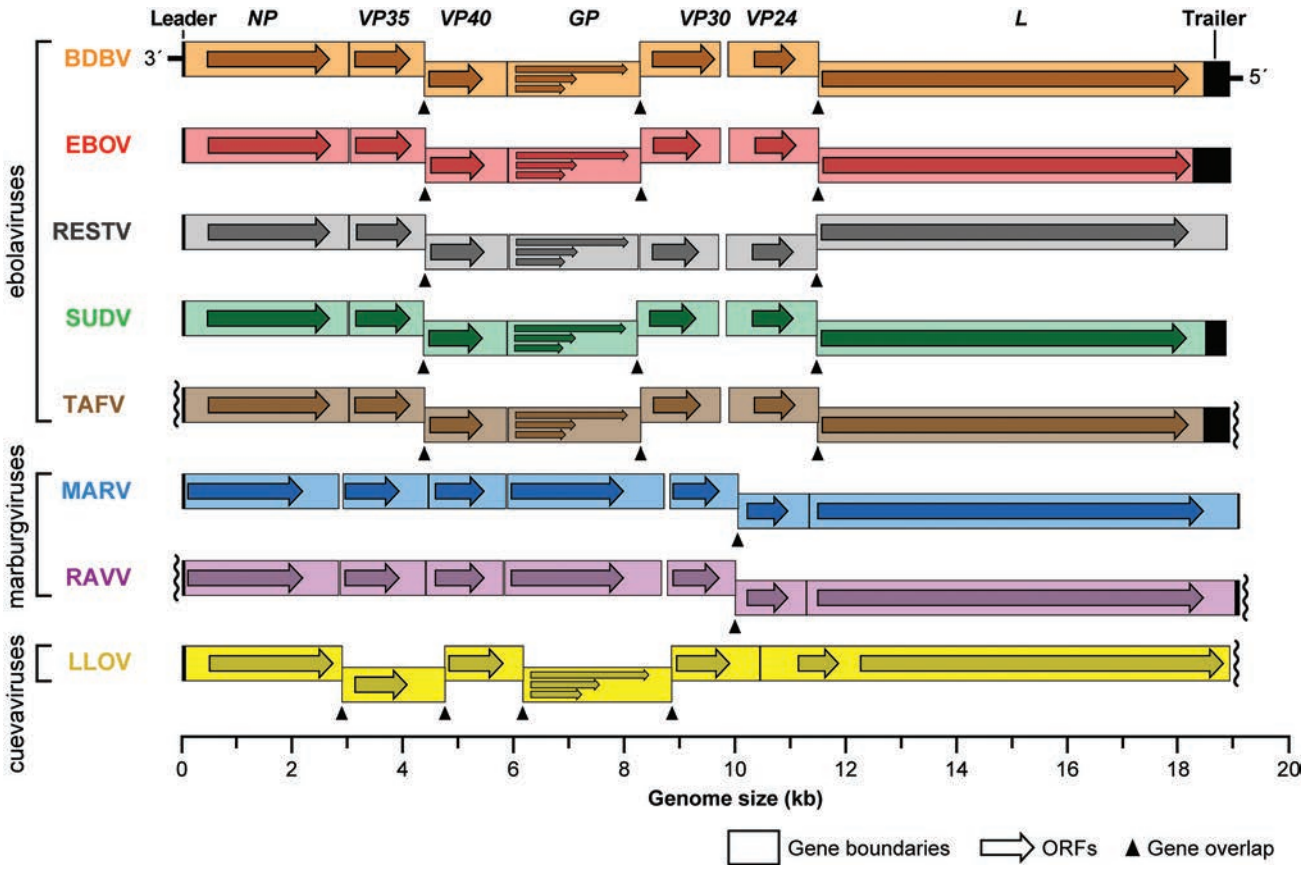

Figure 23-3. Filovirus genome organization. All filovirus genomes have the same overall sequence of genes (*rectangles*) and open reading frames (*horizontal arrows*), but differ from each other in the number and position of gene overlaps (*triangles*) and intergenic regions. Cuevaviruses and ebolaviruses differ from marburgviruses in that their GP genes contain three—rather than one—open reading frames that are accessible through transcriptional editing. Cuevaviruses differ from ebolaviruses and marburgviruses in that VP24 and L open reading frames are transcribed from a single bicistronic transcript. Genomes are drawn to scale; *waved lines* indicate incomplete sequencing of 3′ and 5′ leader and trailer sequences. See Figure 23-1 for color explanations. ORF: open reading frame

host has been unambiguously identified. Both viruses could be isolated repeatedly from several wild and seemingly healthy Egyptian rousettes (cavernicolous and frugivorous pteropodid bats of the species *Rousettus aegyptiacus*) inhabiting Kitaka Cave and Python Cave in Uganda.[60,61] A few human MVD cases were recorded among visitors of these caves.[60,61] Studies suggest that low-level transmission of both viruses among these bats occurs throughout the year with peaks of infection in older juveniles.[60] Experimental infections of Egyptian rousettes demonstrated their capacity for oral shedding of MARV,[62] suggesting that half-eaten and thereby contaminated fruit could be part of a bat–human transmission route. However, Egyptian rousettes are widely distributed across sub-Saharan and Northern Africa and Western and Southern Asia in colonies reaching up to 50,000 bats. Consequently, it is puzzling why MVD outbreaks among humans are rare events that seem to be confined to a few geographic zones of Africa.[63]

Only a loose association with bats is indicated in the cases of EBOV and RESTV. For instance, anti-EBOV immunoglobulin G antibodies or extremely short (≈300 nt) EBOV genomic fragments were detected in individual bats of several pteropodid species collected in Gabon, Ghana, and Republic of the Congo, but never both at the same time.[64–67] Anti-RESTV immunoglobulin G was detected in pteropodid bats sampled in the Philippines.[68] However, neither replicating isolates nor coding-complete genomes have yet been recovered from any bat, which is puzzling given that filoviruses generally replicate to high titers in standard cell cultures.[63] Potentially, these bats have only been exposed to, rather than infected with, EBOV/RESTV by a yet unidentified host. Finally, any connection to healthy bats is lacking for BDBV, LLOV, SUDV, and TAFV.[63]

TABLE 23-3

FUNCTION OF FILOVIRAL PROTEINS

Protein	Encoding Gene	Protein Characteristics	Protein Function
Nucleoprotein (NP)[1–8]	NP	Second-most abundant protein in infected cells and in virions; consists of two distinct functional modules; homooligomerizes to form helical polymers; binds to genomic and antigenomic RNA, VP35, VP40, VP30, and VP24; phosphorylated; depending on filovirus, *O*-glycosylated and/or sialylated	Major RNP component; nucleocapsid and cellular inclusion body formation; encapsidation of filovirus genome and antigenome; genome replication and transcription
Polymerase cofactor = viral protein 35 (VP35)[2,9–23]	VP35	Homooligomer; phosphorylated; binds to double-stranded RNA, NP, and L	RNP component; Replicase-transcriptase cofactor; inhibits innate immune response by interfering with MDA-5 and RIG-1 pathways, IRF-3 and IRF-7, and the RNAi pathway
Matrix protein = viral protein 40 (VP40)[11,12,24–26,27–42]	VP40	Most abundant protein in infected cells and in virions; consists of two distinct functional modules; homooligomerizes to form dimers and circular hexamers and octamers; binds single-stranded RNA, α-tubulin, VP35; hydrophobic; membrane-associated; contains one (marburgviruses) or three (cuevaviruses and ebolaviruses) late-budding motifs; binds NEDD4 and Tsg101; ubiquitinylated	Matrix component; regulation of genome transcription and replication; regulation of virion morphogenesis and egress; sequence determines filovirus pathogenicity in rodents. Marburgviruses only: inhibits innate immune response by JAK1 signaling
Cuevaviruses and ebolaviruses only: secreted glycoprotein (sGP)[43–46]	GP	Mostly nonstructural; secreted as a parallel homodimer in high amounts from infected cells; *N*-glycosylated, C-mannosylated, sialylated	Unknown. Hypothesized to be an antibody-decoy and antiinflammatory agent
Glycoprotein ($GP_{1,2}$)[47–60]	GP	Type 1 transmembrane and class I fusion protein; cleaved to GP1 and GP2 subunits that heterodimerize; mature protein is a trimer of $GP_{1,2}$ heterodimers; inserts into membranes; heavily *N*- and *O*-glycosylated, acylated, phosphorylated. Tumor necrosis factor α-converting enzyme (TACE) converts $GP_{1,2}$ into a soluble form ($GP_{1,2\Delta}$)	Virion adsorption to filovirus-susceptible cells via cellular attachment factors; determines filovirus cell and tissue tropism; induction of virus-cell membrane fusion subsequent to endolysosomal binding to NPC1; inhibits innate immune response by interfering with tetherin. Function of $GP_{1,2\Delta}$ is unknown
Cuevaviruses and ebolaviruses only: secondary secreted glycoprotein (ssGP)[61]	GP	Nonstructural; secreted as a glycosylated monomer	Unknown
Cuevaviruses and ebolaviruses only: Δ-peptide[62–64]	GP	Nonstructural; secreted; largely unstructured; *O*-glycosylated and sialylated	Unknown. Hypothesized to act as a suppressor of filoviral superinfection and/or as a viroporin
Transcriptional activator = viral protein 30 (VP30)[65–75]	VP30	Hexameric zinc finger protein; binds single-stranded RNA, NP, and L; phosphorylated	RNP component Cuevaviruses and ebolaviruses only: transcription initiation, reinitiation, and antitermination

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Table 23-3 *continued*

Secondary matrix protein = viral protein 24 (VP24)[66,68,76–86]	VP24	Homotetramerizes; hydrophobic and membrane-associated	Matrix component; regulation of genome transcription and replication; regulation of virion morphogenesis and egress; sequence determines filovirus pathogenicity in rodents Cuevaviruses and ebolaviruses only: Blocks phosphorylation of MAPK and prevents karyopherin shuttling from cytoplasm into the nucleus; inhibits host-cell signaling downstream of IFN-$\alpha/\beta/\gamma$
RNA-dependent RNA polymerase = large protein (L)[2,23,67,87–91]	L	Homodimerizes; binds to genomic and antigenomic RNA, VP35, and VP30; contains ATP-binding sites and a cap-1 MTAse domain	RNP component; genome replication and transcription; transcriptional editing

ATP: adenosine triphosphate
IFN: interferon
IRF: interferon regulatory factor
JAK1: Janus kinase 1
MAPK: mitogen-activated protein kinase
MDA-5: melanoma differentiation-associated protein-5
MTAse: methyltransferase
NEDD4: neural precursor cell-expressed, developmentally down-regulated protein 4
NPC1: Niemann-Pick C1 protein
RIG-1: retinoic acid-inducible gene-1
RNA: ribonucleic acid
RNAi: RNA interference
RNP: ribonucleoprotein complex
Tsg101: tumor susceptibility gene 101 protein
Data sources: (1) Becker S, Rinne C, Hofsäss U, Klenk HD, Mühlberger E. Interactions of Marburg virus nucleocapsid proteins. *Virology*. 1998;249:406–417. (2) Mühlberger E, Lötfering B, Klenk HD, Becker S. Three of the four nucleocapsid proteins of Marburg virus, NP, VP35, and L, are sufficient to mediate replication and transcription of Marburg virus-specific monocistronic minigenomes. *J Virol*. 1998;72:8756–8764. (3) Sanchez A, Kiley MP, Klenk HD, Feldmann H. Sequence analysis of the Marburg virus nucleoprotein gene: comparison to Ebola virus and other non-segmented negative-strand RNA viruses. *J Gen Virol*. 1992;73(Pt 2):347–357. (4) Lötfering B, Mühlberger E, Tamura T, Klenk HD, Becker S. The nucleoprotein of Marburg virus is target for multiple cellular kinases. *Virology*. 1999;255:50–62. (5) Kolesnikova L, Mühlberger E, Ryabchikova E, Becker S. Ultrastructural organization of recombinant Marburg virus nucleoprotein: comparison with Marburg virus inclusions. *J Virol*. 2000;74:3899–3904. (6) Huang Y, Xu L, Sun Y, Nabel GJ. The assembly of Ebola virus nucleocapsid requires virion-associated proteins 35 and 24 and posttranslational modification of nucleoprotein. *Mol Cell*. 2002;10:307–316. (7) Noda T, Hagiwara K, Sagara H, Kawaoka Y. Characterization of the Ebola virus nucleoprotein-RNA complex. *J Gen Virol*. 2010;91(Pt 6):1478–1483. (8) Dziubanska PJ, Derewenda U, Ellena JF, Engel DA, Derewenda ZS. The structure of the C-terminal domain of the Zaire ebolavirus nucleoprotein. *Acta Crystallogr D Biol Crystallogr*. 2014;70(Pt 9):2420–2429. (9) Cárdenas WB, Loo YM, Gale M Jr, et al. Ebola virus VP35 protein binds double-stranded RNA and inhibits alpha/beta interferon production induced by RIG-I signaling. *J Virol*. 2006;80:5168–5178. (10) Kimberlin CR, Bornholdt ZA, Li S, Woods VL Jr, MacRae IJ, Saphire EO. Ebolavirus VP35 uses a bimodal strategy to bind dsRNA for innate immune suppression. *Proc Natl Acad Sci U S A*. 2010;107:314–319. (11) 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. (12) Bukreyev AA, Volchkov VE, Blinov VM, Netesov SV. The VP35 and VP40 proteins of filoviruses. Homology between Marburg and Ebola viruses. *FEBS Lett*. 1993;322:41–46. (13) Basler CF, Wang X, Mühlberger 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. (14) Feng Z, Cerveny M, Yan Z, He B. The VP35 protein of Ebola virus inhibits the antiviral effect mediated by double-stranded RNA-dependent protein kinase PKR. *J Virol*. 2007;81:182–192. (15) Haasnoot J, de Vries W, Geutjes EJ, Prins M, de Haan P, Berkhout B. The Ebola virus VP35 protein is a suppressor of RNA silencing. *PLoS Pathog*. 2007;3:e86. (16) Johnson RF, McCarthy SE, Godlewski PJ, Harty RN. Ebola virus VP35-VP40 interaction is sufficient for packaging 3E-5E minigenome RNA into virus-like particles. *J Virol*. 2006;80:5135–5144. (17) Leung DW, Prins KC, Borek DM, et al. Structural basis for dsRNA recognition and interferon antagonism by Ebola VP35. *Nat Struct Mol Biol*. 2010;17:165–172. (18) Luthra P, Ramanan P, Mire CE, et al. Mutual antagonism between the Ebola virus VP35 protein and the RIG-I activator

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Table 23-3 *continued*

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(**Table 23-3** *continues*)

Table 23-3 *continued*

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Epizootiology of Filoviruses

Filoviruses are highly virulent pathogens for humans. Experimentally, most of them also cause frequently fatal infections in all thus-far studied nonhuman primates (NHPs): common marmosets (*Callithrix jacchus*), crab-eating macaques (*Macaca fascicularis*), grivets (*Chlorocebus aethiops*), hamadryas baboons (*Papio hamadryas*), rhesus monkeys (*Macaca mulatta*), and common squirrel monkeys (*Saimiri sciureus*). After serial laboratory adaptation of filoviruses, rodents such as laboratory mice, guinea pigs (*Cavia porcellus*), and Syrian golden hamsters (*Mesocricetus auratus*) infected with adapted filoviruses can develop fatal infections.[2,69,70] However, whether filoviruses are also natural pathogens for animals other than humans remains under discussion.

Five filoviruses have been loosely associated with epizootics. MARV was discovered in 1967 in West Germany among sick and dying laboratory workers who had used captive grivets imported from Uganda for poliomyelitis vaccine development.[71] However, it was never clarified at what point these monkeys became infected in captivity (Uganda or en route to West Germany) or before capture.[72] No evidence indicates that grivets are infected with filoviruses in the wild.

RESTV was discovered in 1989 when crab-eating macaques coinfected with a simian arterivirus (*Arteriviridae*: *Arterivirus*) were imported from the Philippines into the United States, fell sick, and died.[73] Similar epizootics among captive crab-eating macaques imported from the same Philippine facility occurred in the United States in 1990 and 1996, and in Italy in 1992.[74] In 2008, RESTV was identified in the Philippines in captive domestic pigs (*Sus scrofa*) coinfected with another arterivirus, porcine reproductive and respiratory disease syndrome virus. These pigs suffered and died from a respiratory and abortion disease.[75] It remains unclear how RESTV was introduced into these animal populations and why only four such introductions occurred. Although domestic pigs can be infected experimentally with EBOV[76,77] and experimentally infected piglets can transmit EBOV directly to cohoused crab-eating macaques,[78] evidence of natural infection of wild suids with filoviruses is lacking.

Indirect evidence for natural nonhuman animal filovirus infections exists for EBOV and LLOV. In the case of EBOV, catastrophic declines of central chimpanzee (*Pan troglodytes troglodytes*) and western lowland gorilla (*Gorilla gorilla gorilla*) populations correlated with EBOV-caused EVD epidemics in Gabon and in the Republic of the Congo.[79–81] In addition, duiker (*Cephalophus* spp) populations seem to have been affected at the same time. However, supporting evidence of EBOV involvement in these epizootics is limited to polymerase chain reaction (PCR)-based detection of short genomic fragments and detection of antigen in three central chimpanzees, 10 western lowland gorillas, and one duiker.[81] Replicating EBOV isolates have not yet been obtained, and complete or coding-complete EBOV genomes have yet to be detected to directly prove animal infection and possibly a link between human and animal disease.

LLOV, however, was discovered in wild animals. A coding-complete LLOV genome was assembled from tissues taken from insectivorous Schreibers' long-fingered bats (*Miniopterus schreibersii*). These bats were among hundreds that died of an unknown cause in 2002 in Cueva del Lloviu in Spain.[82] However, in the absence of a replicating LLOV isolate, determining whether LLOV caused the bat fatalities or whether the bats were infected subclinically with the virus and died of different causes is impossible.

The only direct evidence for filovirus infection of animals in the wild exists for TAFV. In 1994, a viral hemorrhagic fever-like epizootic killed most members of a wild western chimpanzee (*Pan troglodytes verus*) community in Taï National Park in Côte d'Ivoire (Western Africa).[83] A female ethologist accidentally infected herself with the viral-hemorrhagic-fever-causing pathogen while performing necropsies on the deceased animals. TAFV was isolated from clinical material, and serological testing demonstrated that western chimpanzees were infected with the same agent.[84] It is unclear, however, how the chimpanzees became infected and whether such infections are common or unusual events.

Epidemiology of Filovirus Infections

Filoviruses were discovered in 1967 in West Germany.[71] Since then, 37 human EVD and MVD outbreaks have been recorded (Figure 23-4).[85] The incidence of MVD and EVD apparently has continued to increase over the years, but this increase may simply result from improved surveillance and reporting. Statistical support that any ebolavirus is more virulent than another is scant, although based on current case numbers, MVD appears to be more lethal compared to EVD (Figure 23-4). Close to all of the 37 filovirus disease outbreaks occurred in Middle/Eastern Africa. Interestingly, "hot spots" for filovirus disease outbreaks seem to exist. For instance, EBOV reappears continuously in Gabon, Republic of the Congo, and western Democratic Republic of the Congo; whereas BDBV, SUDV, and MARV caused repeated outbreaks in the northeastern Democratic Republic of the Congo, southern South Sudan, and Uganda (Figure 23-5).

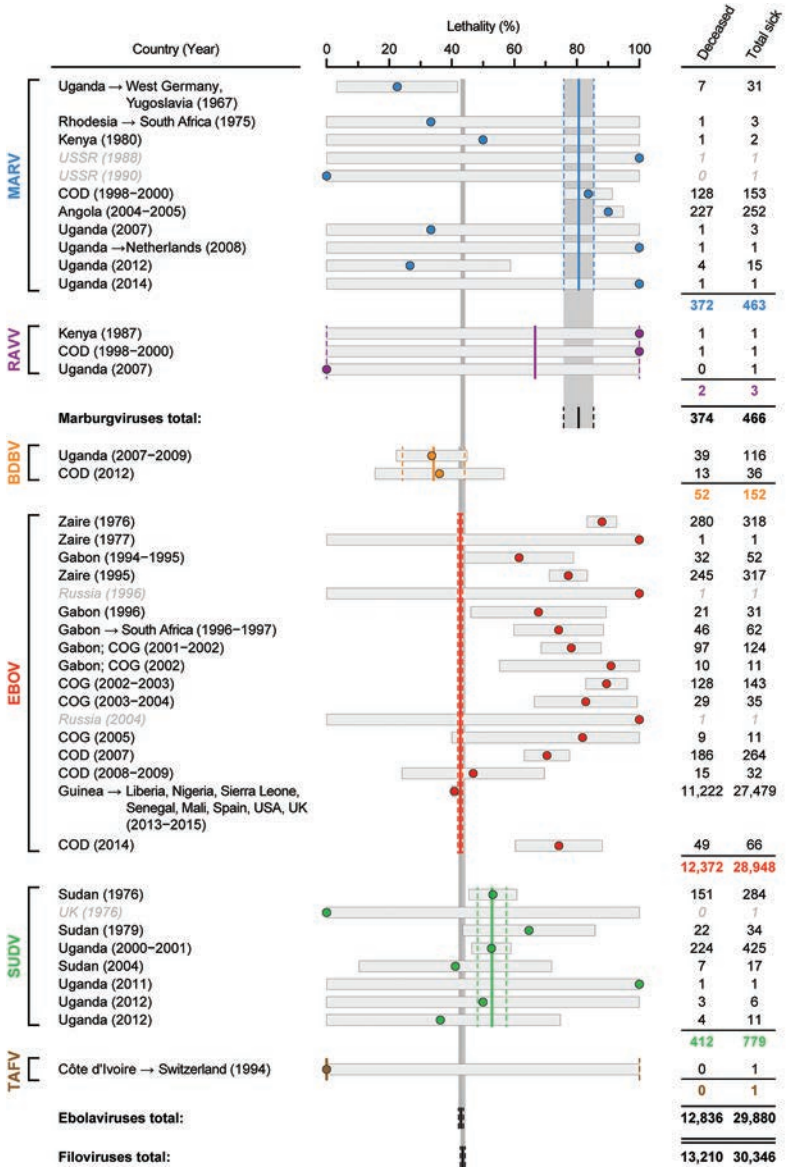

Figure 23-4. Ebola and Marburg virus disease outbreaks. Ebola virus disease and Marburg virus disease outbreaks are listed chronologically by virus (*colored vertically on the left*). International case exportations are pointed out by *arrows*; proven laboratory infections are highlighted in *gray and italics*. Total case numbers and total number of fatalities are itemized for each outbreak in the utmost right columns (updated from data sources 1 and 2). The lethality/case fatality rate (*dots*) for each outbreak is plotted in the middle column on a 0% to 100% scale along with 99% confidence intervals (*gray horizontal bars*). The average lethality of a particular virus or virus group is shown by *vertical lines* (99% confidence intervals are emphasized by *dashed lines*). The vertical line showing the average lethality of all Ebola virus disease outbreaks overlaps with the vertical line showing the average lethality of all filovirus disease outbreaks and the vertical line showing the average lethality of all disease outbreaks caused only by Ebola virus (*red*). At the time of this writing, the 2013–2015 Ebola virus-caused Ebola virus disease outbreak in Western Africa has not been brought under control. Consequently, the case and fatality numbers are still subject to change and lethality should rather be regarded as a proportion of fatal cases than lethality until final numbers become available. See Figure 23-1 for color explanations. COD: Democratic Republic of the Congo; COG: Republic of the Congo; UK: United Kingdom; USSR: Union of Soviet Socialist Republics.

Data sources: (1) Kuhn JH. *Filoviruses: A Compendium of 40 Years of Epidemiological, Clinical, and Laboratory Studies*. In: Calisher CH, ed. *Archives of Virology* Supplementa Series, Vol 20. Vienna, Austria: Springer-Verlag Wien; 2008. (2) Kuhn JH, Dodd LE, Wahl-Jensen V, Radoshitzky SR, Bavari S, Jahrling PB. Evaluation of perceived threat differences posed by filovirus variants. *Biosecur Bioterror*. 2011;9:361–371. (2) Kuhn JH. Ebolavirus and Marburgvirus infections. In: Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J, eds. *Harrison's Principles of Internal Medicine*. Vol 2. 19th ed. Columbus, OH: McGraw-Hill Education; 2015:1323–1329.

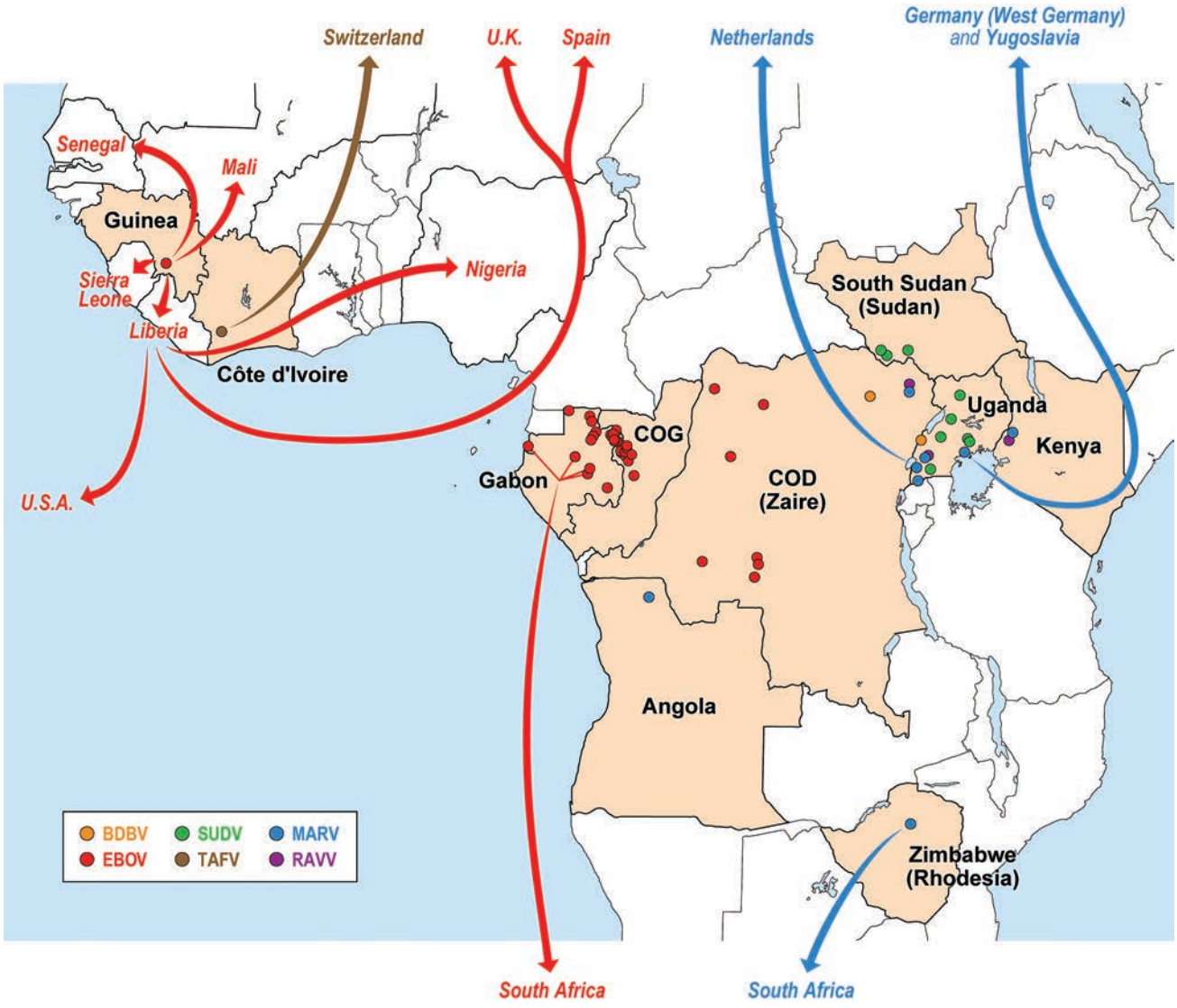

Figure 23-5. Ebola and Marburg virus disease outbreaks. Middle/equatorial African countries affected by Ebola virus disease and/or Marburg virus disease outbreaks are shown in *light brown* with outbreak locations marked as dots colored according to the etiological filovirus (updated from data sources 1 and 2). *Arrows* mark international case exportation. Former country names are listed in parentheses under the present name.
COD: Democratic Republic of the Congo; COG: Republic of the Congo.
Data sources: (1) Kuhn JH. *Filoviruses: A Compendium of 40 Years of Epidemiological, Clinical, and Laboratory Studies*. In: Calisher CH, ed. *Archives of Virology* Supplementa Series, Vol 20. Vienna, Austria: Springer-Verlag Wien; 2008. (2) Kuhn JH, Dodd LE, Wahl-Jensen V, Radoshitzky SR, Bavari S, Jahrling PB. Evaluation of perceived threat differences posed by filovirus variants. *Biosecur Bioterror*. 2011;9:361–371. (3) Kuhn JH. Ebolavirus and Marburgvirus infections. In: Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J, eds. *Harrison's Principles of Internal Medicine*. Vol 2. 19th ed. Columbus, OH: McGraw-Hill Education; 2015:1323–1329.

Almost all filovirus disease outbreaks began with a single introduction of a filovirus into an index case who subsequently transmitted the infection to other humans. Thus, initial human filovirus infections are extremely rare events and occurred probably less than 50 times since 1967.[2] In general, past filovirus disease outbreaks occurred in rural and often secluded areas and affected only several dozens to a few hundred people.[2] However, a few outbreaks occurred in populated areas, such as the 1995 EVD outbreak caused by EBOV in Kikwit (Zaire) and the 1998–2000 MVD outbreaks from MARV and RAVV around Durba and Watsa, Democratic Republic of the Congo (the former Zaire). This pattern shifted dramatically in December

2013 when the largest EVD outbreak began in Western Africa from a single introduction of EBOV.[86,87] As of October 11, 2015, this outbreak has thus far caused 28,490 cases and 11,312 deaths in Guinea, Liberia, Mali, Nigeria, Senegal, and Sierra Leone (Figure 23-4).

Serological Surveys

Numerous serological surveys for antibodies against filoviral antigens have been performed in human and animal populations to further define the geographic spread of filoviruses and to better estimate risk of infection.[2] However, results of most of these surveys are puzzling. In some surveys, the seroprevalence of anti-EBOV antibodies is extremely high (>5%–20%) in humans indicating frequent exposure to EBOV or related agents in the absence of disease. In other surveys, the seroprevalence of antifilovirus antibodies is moderate among humans living in areas that never had filovirus disease outbreaks (eg, certain African countries, Belarus, Germany, Ukraine). Many of these studies used MARV, EBOV, or SUDV antigens in indirect immunofluorescence assays (IFAs), which are subjective and thus difficult to interpret. IFA serosurveys are therefore regarded as presumptive by most experts. Modern serosurveys rely on the use of enzyme-linked immunosorbent assays (ELISAs) in conjunction with confirmatory western blot for the detection of antifilovirus antibodies. Few such studies were published, and results of these studies most often did not confirm IFA results.[2]

Overall, three disparate possibilities arise from the performed serosurveys (IFA and/or ELISA). First, all obtained results may be artifacts based on common nonfilovirus antibodies in human sera that are cross-reactive with the used filoviral antigens, thereby leading to false-positive results. Second, filoviruses could subclinically infect humans or cause only mild disease, thereby leading to high seropositivity rates. Current data on the possibility of such infections are scarce[88,89] and hotly debated, but the currently ongoing EVD outbreak in Western Africa may reveal subclinical infections resulting from the sheer number of recorded infections. Third, the discovery of LLOV in Spain[82] indicates the possibility that filovirus diversity and geographic distribution is broader than currently appreciated. Perhaps contact with possibly nonpathogenic filoviruses (eg, LLOV- or RESTV-related viruses) induces antibodies that are cross-reactive with closely related filoviral antigens. Without convincing data for any of these possibilities, serosurvey data should not be ignored, but they should be used with caution for prediction of filovirus distribution or infection risk assessments.

Environmental Niche Modeling

Environmental niche modeling (ie, the use of algorithms to predict the geographic distribution of organisms on the basis of their environmental distribution using meteorological and other data) indicates succinct distributions for filoviruses in the Afrotropic ecozone.[90–95] According to these models, ebolaviruses are endemic in humid rain forests in Western and Middle Africa and South-Eastern Asia, whereas marburgviruses circulate in caves located in arid woodlands in Middle, Eastern, and Southern Africa.[90,91] Filovirus emergence in human populations appears to be associated with the appearance of climate anomalies or drastic climate changes.[92] For instance, ebolavirus activity is suggested to be correlated with unusually heavy rainfalls subsequent to extended dry periods.[90,94,95] If these models prove correct, then filovirus disease outbreaks should be expected in numerous African countries that have thus far not experienced (or noticed) any outbreaks.[93]

TRANSMISSION

As the natural reservoir hosts for most filoviruses are unknown, how filoviruses are introduced into the human population is unclear. Researchers are tempted to speculate that initial infections occur after direct contact with tissues, secretions, or excretions of an animal or after a bite or sting.[96,97] Even in the case of human infections in Ugandan caves that harbored MARV- and RAVV-infested Egyptian rousettes,[60,61] it remains to be explained how these few people became infected, and why many others who visited these caves did not.

Human-to-human spread of filovirus infections is better understood. Epidemiological studies clearly demonstrate that filovirus transmission almost exclusively occurs through direct person-to-person contact or through direct contact with filovirus-contaminated material.[2,98,99] Airborne spread has not been demonstrated for any filovirus during a natural outbreak, although healthcare workers risk infection during artificial aerosol creation performed as part of medical procedures such as centrifugation of samples, intubation of patients, or suction used during surgical procedures.[100,101]

Filoviruses replicate in humans to high titers (>10^6 plaque-forming units/mL) and at least in the case of EBOV, vast quantities of antigen deposit in the skin and around skin appendages.[102] In animal models, the LD_{50} of EBOV has been estimated to be as low as 1 plaque-forming unit.[103] As a consequence, filoviruses

are highly infectious and readily contagious through close contact with skin or mucous membranes, especially in the presence of small lesions.[104–107] Filoviruses or filovirus RNA may be present in genital, nasal, and other bodily secretions. Transmission appears to be a rare event during the early, asymptomatic phase of disease.[100,101] However, in the absence of personal protective equipment (PPE; disposable gowns, gloves, shoe covers, face-shields, or goggles), transmission occurs readily. Transmission is typical between sick people and their family members, friends, or healthcare workers who care for them; between deceased people and people who prepare bodies for funerals; and between medical personnel who handle medical samples, contaminated medical equipment, or decontamination. A second important transmission pathway is nosocomial spread through contaminated and reused disposable needles and syringes that, unfortunately, is still common in many chronically underfunded and therefore underequipped African hospitals.[100,101,104,108–111] Implementation of quarantine measures and use of proper PPE usually suffice to interrupt human-to-human transmission and to terminate outbreaks.[100,101,105,109,110,112]

At this time, the question remains whether filoviruses truly adapt to the human host during prolonged interhuman transmission, and whether such adaptation could result in the natural selection of variants that is either more or less transmissible or more or less virulent. A recent study performed during the 2013–2015 EVD outbreak in Africa indicates that mutations accumulate and particular subpopulations of EBOV arise during transmission,[87] but these subpopulations have not been associated with particular phenotypes.

THREAT TO THE WARFIGHTER

The warfighter could potentially be at risk of filovirus infection during humanitarian deployment, military campaigns, or war. Exposure to filoviruses could occur coincidentally through contact with unknown filovirus reservoir(s) and accidentally through exposure to infected people, deceased patients, or materials contaminated with human secretions or excretions. In addition, the warfighter may be exposed deliberately during an attack with biological weapons deployed by terrorists, hostile groups, or nation states.[113] Coincidental and accidental risks can be dramatically reduced for the warfighter if common sense practices for tourists and standard operating procedures for healthcare workers are implemented.

Filoviruses and the Soviet Biological Warfare Program

The Soviet Union maintained a highly clandestine biological weapons program from at least 1918 until at least 1991.[114,115] In 1999, a published account from a high-ranking defector of the civilian "Biopreparat" arm of this program revealed that two filoviruses, EBOV and MARV, were included in the program.[115] Additional revelations about the biological weapons program are scarce. Consequently, knowledge of the scope, goals, and achievements of especially the second generation of the program (1972–1991) is deduced from accounts from several additional defectors and a few researchers who were previously involved and legally left the Soviet Union/Russia, as well as from a few leaked classified reports or memos.[114]

Classified filovirus research probably began in the Soviet Union shortly after the discovery of MARV in 1967 in Marburg and Frankfurt, West Germany, and Belgrade, Yugoslavia. West German and Yugoslavian scientists provided several isolates of the novel virus (most notably MARV Popp) to numerous international institutes for characterization studies to counter allegations that West Germany had developed a biological weapon. Among these institutes was the Union of Soviet Socialist Republics Academy of Medical Sciences Scientific Research Institute of Poliomyelitis and Viral Encephalitides (now named the M.P. Chumakov Institute of Poliomyelitis and Viral Encephalitides of the Russian Academy of Medical Sciences) in Moscow.[114,116] Unclassified, nonmilitary-related research began at the institute immediately and resulted in a few published reports in Russian from 1968 to 1972.[2] Current thinking is that filovirus research became classified thereafter and soon was abandoned at that institute after MARV cultures were transferred to the main military virology institute, the Scientific Research Institute of Sanitation of the Union of Soviet Socialist Republics Ministry of Defense in Zagorsk (now named the Virology Center under the Scientific-Research Institute of Microbiology in the renamed city of Sergiev Posad) close to Moscow.[114]

The Institute of Tropical Medicine in Antwerp, Belgium, which had received MARV during the 1967 MVD outbreak and EBOV during the 1976 EVD outbreaks, provided MARV isolate Voege and an EBOV Yambuku isolate in the mid-1980s within a standard collaboration for diagnostics development to the Belorussian Scientific-Research Institute for Epidemiology and Microbiology of the Belorussian SSR Ministry of Health (now named the Republican Research and Practical Center for Epidemiology and Microbiology) in Minsk. Although the institute in Minsk continued international collaboration and published manuscripts

using MARV and EBOV, both viruses were most likely also transported to the institute in Zagorsk.

Probably from Zagorsk, MARV Popp and the EBOV Yambuku isolates were transferred to the highly secretive Scientific-Production Association "Vector" (now named the State Scientific Center for Virology and Biotechnology "Vector") in the closed settlement of Kolcovo close to Novosibirsk. MARV Voege and the same EBOV isolate were also transferred to the Scientific-Research Anti-Plague Institute for Siberia and the Far East in Irkutsk (Figure 23-6).[114] Judging from Russian publications released in the mid-1990s, the three institutes in Irkutsk, Kolcovo, and Zagorsk made significant progress in basic research in terms of EBOV

Figure 23-6. Locations of clandestine filovirus research in the Soviet Union. Marburg virus was provided to the Union of Soviet Socialist Republics Academy of Medical Sciences Scientific Research Institute of Poliomyelitis and Viral Encephalitides in Moscow by West German and Yugoslavian scientists and then transported to the Scientific-Research Institute of Sanitation of the Union of Soviet Socialist Republics Ministry of Defense in Zagorsk. Belgian scientists provided Marburg virus and Ebola virus to the Belorussian Scientific-Research Institute for Epidemiology and Microbiology of the Belorussian SSR Ministry of Health, which also forwarded cultures to Zagorsk. From there, cultures were transferred to the Scientific-Production Association "Vector" in Kolcovo and the Scientific-Research Anti-Plague Institute for Siberia and the Far East in Irkutsk. Major offensive research and development most likely occurred in Zagorsk and Kolcovo at least until 1991.
Data sources: (1) Zilinskas RA, Leitenberg M, with Kuhn J. *The Soviet Biological Weapons Program: A History*. Cambridge, MA: Harvard University Press; 2012. (2) Alibek K, Handelman S. *Biohazard—The Chilling True Story of the Largest Covert Biological Weapons Program in the World—Told from Inside by the Man Who Ran It*. New York, NY: Random House; 1999. (3) Zilinskas RA. The anti-plague system and the Soviet biological warfare program. *Crit Rev Microbiol*. 2006;32:47–64.

and MARV genomic sequencing, established ELISA- and PCR-based diagnostics, and developed parenteral and aerosol rodent and NHP MVD/EVD models.[114]

Little is known about bona fide offensive research and development efforts. Based on current data, EBOV research apparently did not progress beyond the research stage because of production problems.[114] MARV, however, was specified in the Soviet 11th five-year plan (1981–1985) to be weaponized.[114] Weaponization efforts probably began in 1983 at the Scientific-Production Association or "Vector," focusing on characterization of the pathogen, high-titer production in rodents and later in tissue bioreactors, and production of dried and milled formulations. Finally, these formulations were tested in aerosol experiments using animals around 1991. However, most likely few or none of the formulations reached the validation stage, and type-classified weapons (ie, MARV-loaded weapons that had been tested and succeeded in open-air testing) were not developed.[114]

The extensive effort within the Soviet biological weapons program and the ultimate failure to produce a reliable weapon indicate that the risk of attack with a biological weapon constructed to spread filoviruses is relatively low, but not negligible. Although filoviruses are not naturally airborne and transmission from person-to-person is negligible without direct contact, the Soviet program suggests that these hurdles are thought by some not to be unsurmountable. However, a large-scale attack on civilians or armed forces with filoviruses seems unlikely and possible only by nation states rather than by small adversary groups. Such groups could—in theory—attempt to introduce filoviruses into human populations by means other than weaponry (eg, direct injection with needles; self-infection) to induce panic and thereby affect the economy of target populations.[113,117] Therefore, educating the general public about filoviruses is vital to reduce the psychological impact of such an attack.

PREVENTION

Behavioral Modification

Prevention of initial introduction of filoviruses into human or other animal populations is difficult to impossible as long as the ecology of the viruses is not understood and their natural host reservoirs remain unknown. However, general "good infection control behaviors" should be encouraged to minimize the risk of initial infection. Such control behaviors include the avoidance of direct contact with wild animals; consumption of uncooked or undercooked wildlife; and unprotected exposure to animal excretions, secretions, fluids, or tissues. Control behaviors further include consuming water that has been boiled, reducing contact with arthropods (eg, application of insect repellents, using mosquito nets, screening for ticks), avoiding contact with obviously sick people, and avoiding unprotected sex. During a filovirus disease outbreak, locals should be educated about the nature of filoviruses. Certain cultural practices, such as handshaking, or particular funeral rituals, such as ritual hand washing or embalming of bodies, should either be strongly discouraged or modified to decrease filovirus transmission risk.[118–121] Quarantine of infected people and avoidance of direct person-to-person contact generally suffices to prevent further spread. Healthcare and other staff should don proper PPE before handling patients or suspected cases of filovirus infection, clinical samples, or potentially contaminated material. Strict implementation of barrier nursing techniques in patient care is also vital. N-95/N-100 and positive air pressure respirators, if available, should be used especially during clinical procedures that may generate aerosols. However, users should be aware that positive air pressure respirators may induce fear, especially among local populations. As fomites are an important route of filovirus transmission, reuse of medical equipment should be avoided whenever possible. At all times, disposables should be used only once and promptly discarded.[100,112–127]

Filovirus Inactivation and Decontamination

Filoviruses produce enveloped virions that contain single-stranded RNA genomes.[26,27] These virions, which are relatively labile, are rapidly inactivated by heat, pressure, radiation, or contact with detergents. Cheap and commonly available detergents (diethyl ether, phenolic compounds, sodium deoxycholate) and oxidizing agents, such as bleach or bleaching powder, should be used to disinfect surfaces or patient excreta or secreta.[128–132] However, despite their overall lability, filovirions are stable for several days in liquids such as drying blood and on surfaces typically found in treatment units for more than 5 days.[133] Corpses, which may contain infectious filoviruses for extended periods of time,[134] should be buried quickly. Ideally, potentially contaminated disposables are autoclaved, irradiated, or burned.

Vaccines

Despite numerous and diverse efforts,[2,135–138] no FDA-approved vaccine exists to prevent filovirus infections. Candidate vaccines include inactivated and attenuated filoviruses, subunit vaccines (adenovirus,[139–145] alphavirus,[146–150] lyssavirus,[151,152] orthopoxvirus,[153] paramyxovirus,[154–156] and vesiculovirus[157–162] vectors expressing filovirus NP, VP35, VP40, $GP_{1,2}$, VP24, VP30, and/or VP24), naked DNA vaccines encoding filovirus proteins (alone or in combination with adenovirus-based vectors),[140,163–166] and filovirus-like particles consisting only of VP40, NP, and $GP_{1,2}$.[167–170] These candidate vaccines were variably efficacious in different animal models. All of these vaccines have advantages and disadvantages in regard to safety profiles, induction of long-term immune responses, or ease of production.[2,135–138] In recent years, consensus has been reached that only platforms that are highly protective in NHP models of filovirus disease should be considered for further development.[69] Among these platforms, the most promising candidate vaccines are those that have been built using adenoviral or vesiculoviral backbones or filovirus-like particles (Table 23-4).

DISEASE

EVD and MVD are largely characterized through clinical observation of patients in underequipped hospitals,[171–178] individual observations of patients who were transported to developed countries or suffered from accidental infections,[179–183] limited examination of tissues obtained during human outbreaks via biopsies,[102,112,184] and a very low number of often incomplete autopsies.[185–192] Most of the examinations, including biopsies and autopsies, were performed before techniques for characterization of molecular pathogenesis events were available. Consequently, a paucity of EVD and MVD biomarkers exists.[193,194] Given the rarity of EVD and MVD outbreaks, disease characterization has therefore depended on the use of filovirus-susceptible animals (rodents and NHPs). Although frequently referred to as "models" of EVD and MVD, the human disease remains largely uncharacterized, and animal infections do not necessarily mimic EVD and MVD completely.[195] For instance, hemorrhagic manifestations, disseminated intravascular coagulopathy, bystander apoptosis, and lethality differ among the types of animals used as well as humans.

Wild type filoviruses do not cause disease in rodents and therefore require serial adaptation to produce disease in these rodents.[103,196] Such adaptation has been challenging, especially with laboratory mice. Consequently, true mouse models are only available for three filoviruses (ie, EBOV, MARV, RAVV).[103,197–199] Laboratory mice are frequently used for initial MCM evaluation efforts for many reasons including the following:

- Ethical concerns are limited about such experiments.
- Mice are easily maintained.
- Experiments are possible involving large numbers of animals.
- The clonal background of laboratory mice simplifies statistical analysis of observed MCM effects on infection.[200]

Golden hamsters are becoming increasingly popular as models for EBOV-induced EVD because they—in contrast to laboratory mice—develop pronounced coagulatory defects mimicking those seen in humans.[201] Guinea pigs are typically used as a bridge between small rodent (laboratory mice, hamsters) and NHP models for infections caused by EBOV and MARV.[196,202–204] In contrast to laboratory mice, MCM evaluation results recorded during guinea pig experiments often translate to similar observations in NHPs. In addition, these experiments are less expensive and not as logistically challenging as NHP experiments.

Nevertheless, NHPs are considered the gold standard for MCM evaluation, which is largely a result of requirements specified in the US Food and Drug Administration's "Animal Rule" for licensure of candidate vaccines and therapeutics that cannot be tested in human clinical trials.[69] EBOV and MARV rapidly infect crab-eating macaques, grivets, hamadryas baboons, rhesus monkeys, and common marmosets and induce a usually uniformly lethal disease.[2,69,205–210] SUDV and RAVV infections can also be studied in crab-eating macaques and rhesus monkeys,[207] whereas experiments using other NHPs have not been reported. Truly useful NHP models for BDBV and TAFV infections have yet to be described.

Pathogenesis

$GP_{1,2}$ embedded in the envelope of filovirions determines cell and therefore tissue tropism of filoviruses based on its interaction with cell surface attachment factors and the intracellular receptor, NPC1.[40] Filoviruses have a broad tropism, that is, the cognate binding partners of $GP_{1,2}$ are expressed on a wide variety of cell types[41] (in vivo, notable exceptions are lymphocytes, myocytes, and neurons[20]). In addition, cuevaviruses and ebolaviruses, but not marburgviruses, produce

TABLE 23-4

SELECTED PROMISING CANDIDATE VACCINES FOR FILOVIRUS INFECTIONS

Candidate Vaccine	Antigen	Efficacy in Nonhuman Primates	Additional Information
Filovirus-like virions (VLPs)[1]	**EBOV**-like virion consisting of **EBOV** NP, VP40, and $GP_{1,2}$	100% survival of crab-eating macaques exposed to **EBOV**	Nonreplicating protein-based vaccine; low safety risks; clinical-grade materials will be required for further development
HPIV-3 vector[2]	**EBOV** $GP_{1,2}$	100% survival of rhesus monkeys exposed to **EBOV**	Replicating, therefore safety concerns; possibly background immunity to vector
Naked DNA + recombinant adenovirus 5 (rAD5) vector[3]	**EBOV** $GP_{1,2}$ + **SUDV** $GP_{1,2}$ (in individual vectors)	Cross protection; 100% survival of crab-eating macaques exposed to **BDBV**	Nonreplicating; possibly background immunity to vector; high dose necessary
Naked DNA + recombinant adenovirus 5 (rAD5) vector[4]	**EBOV** $GP_{1,2}$	100% survival of crab-eating macaques exposed to **EBOV**	Nonreplicating; possibly background immunity to vector; high dose necessary
RABV vector[5]	**EBOV** $GP_{1,2}$	100% survival of rhesus monkeys exposed to **EBOV**	Replicating, therefore safety may be of concern
RABV vector[5]	**EBOV** $GP_{1,2}$	50% survival of rhesus monkeys exposed to **EBOV**	Nonreplicating
Recombinant chimpanzee adenovirus 3 vector (cAD3)[6]	**EBOV** $GP_{1,2}$	100% survival of crab-eating macaques exposed to **EBOV**	Nonreplicating; high dose necessary. Phase 1 clinical trials finished
Recombinant human adenovirus 5 vector (CAdVax)[7]	**EBOV** NP+$GP_{1,2}$ + **MARV** NP+ $GP_{1,2}$ + **RAVV** $GP_{1,2}$ + **SUDV** $GP_{1,2}$ (in individual vectors)	100% survival of crab-eating macaques exposed to **EBOV** or **MARV**	Nonreplicating; possibly background immunity to vector; high dose necessary. **EBOV**-exposed survivors also survived later **SUDV** exposure
Recombinant human adenovirus 5 vector (rAD5)[8,9]	**EBOV** $GP_{1,2}$	100% survival of crab-eating macaques exposed to **EBOV**	Nonreplicating; possibly background immunity to vector; high dose necessary. Phase 1 clinical trials finished
VEEV vector[10]	**EBOV** $GP_{1,2}$ + **SUDV** $GP_{1,2}$ (in individual vectors)	100% survival of crab-eating macaques exposed to **EBOV** or **SUDV**	Nonreplicating; possibly background immunity to vector; clinical-grade materials will be required for further development.
VEEV vector[10]	**SUDV** $GP_{1,2}$	100% survival of crab-eating macaques exposed to **SUDV**	Nonreplicating; possibly background immunity to vector; high dose necessary
Vesicular stomatitis Indiana virus (VSIVΔG) vector[11,12]	**EBOV** $GP_{1,2}$	100% survival of crab-eating macaques exposed to **EBOV**	Replicating, therefore safety may be of concern
Vesicular stomatitis Indiana virus (VSIVΔG) vector[11–14]	**MARV** $GP_{1,2}$	100% survival of crab-eating macaques exposed to **MARV**	Replicating, therefore safety may be of concern

(**Table 23-4** *continues*)

Table 23-4 *continued*

Vesicular stomatitis Indiana virus (VSIVΔG) vector[15]	**BDBV** $GP_{1,2}$	100% survival of crab-eating macaques exposed to **BDBV**	Replicating, therefore safety may be of concern
Vesicular stomatitis Indiana virus (VSIVΔG) vector[16]	**EBOV** $GP_{1,2}$	75% survival of crab-eating macaques exposed to **BDBV**	Replicating, therefore safety may be of concern
Vesicular stomatitis Indiana virus (VSIVΔG) vector[13]	**MARV** $GP_{1,2}$	Cross protection; 100% survival of crab-eating macaques exposed to **RAVV**	Replicating, therefore safety may be of concern
Vesicular stomatitis Indiana virus (VSIVΔG) vector[17]	**EBOV** $GP_{1,2}$ + **MARV** $GP_{1,2}$ + **SUDV** $GP_{1,2}$ (in individual vectors)	100% survival of crab-eating macaques exposed to **EBOV**, **MARV**, **SUDV**, or **TAFV**	Replicating, therefore safety may be of concern
Vesicular stomatitis Indiana virus (VSIVΔG) -vector[17]	**EBOV** $GP_{1,2}$ + **MARV** $GP_{1,2}$ + **SUDV** $GP_{1,2}$ (in individual vectors)	100% survival of rhesus monkeys exposed to **SUDV**	Replicating, therefore safety may be of concern

BDBV: Bundibugyo virus
EBOV: Ebola virus
HPIV3: human parainfluenza virus 3
MARV: Marburg virus
RABV: rabies virus
RAVV: Ravn virus
SUDV: Sudan virus
TAFV: Taï Forest virus
VEEV: Venezuelan equine encephalitis virus
Note: Information on the status of all ongoing filovirus-relevant clinical trials can be found at https://ClinicalTrials.gov with the search terms "Ebola" or "Marburg." See Figure 23-1 for color explanations.
Data sources: (1) Warfield KL, Swenson DL, Olinger GG, Kalina WV, Aman MJ, Bavari S. Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *J Infect Dis*. 2007;196(Suppl 2):S430–S437. (2) Bukreyev A, Rollin PE, Tate MK, et al. Successful topical respiratory tract immunization of primates against Ebola virus. *J Virol*. 2007;81:6379–6388. (3) Hensley LE, Mulangu S, Asiedu C, et al. Demonstration of cross-protective vaccine immunity against an emerging pathogenic ebolavirus Species. *PLoS Pathog*. 2010;6:e1000904. (4) 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. (5) Blaney JE, Marzi A, Willet M, et al. Antibody quality and protection from lethal Ebola virus challenge in nonhuman primates immunized with rabies virus based bivalent vaccine. *PLoS Pathog*. 2013;9:e1003389. (6) Stanley DA, Honko AN, Asiedu C, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nat Med*. 2014;20:1126–1129. (7) Swenson DL, Wang D, Luo M, et al. Vaccine to confer to nonhuman primates complete protection against multistrain Ebola and Marburg virus infections. *Clin Vaccine Immunol*. 2008;15:460–467. (8) Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature*. 2003;424:681–684. (9) 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. (10) Herbert AS, Kuehne AI, Barth JF, et al. Venezuelan equine encephalitis virus replicon particle vaccine protects nonhuman primates from intramuscular and aerosol challenge with ebolavirus. *J Virol*. 2013;87:4952–4964. (11) Geisbert TW, Daddario-Dicaprio KM, Geisbert JB, et al. Vesicular stomatitis virus-based vaccines protect nonhuman primates against aerosol challenge with Ebola and Marburg viruses. *Vaccine*. 2008;26:6894–6900. (12) Jones SM, Feldmann H, Ströher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med*. 2005;11:786–790. (13) Daddario-DiCaprio KM, Geisbert TW, Geisbert JB, et al. Cross-protection against Marburg virus strains by using a live, attenuated recombinant vaccine. *J Virol*. 2006;80:9659–9666. (14) Mire CE, Geisbert JB, Agans KN, et al. Durability of a vesicular stomatitis virus-based Marburg virus vaccine in nonhuman primates. *PLoS ONE*. 2014;9:e94355. (15) Mire CE, Geisbert JB, Marzi A, Agans KN, Feldmann H, Geisbert TW. Vesicular stomatitis virus-based vaccines protect nonhuman primates against Bundibugyo ebolavirus. *PLoS Negl Trop Dis*. 2013;7:e2600. (16) Falzarano D, Feldmann F, Grolla A, et al. Single immunization with a monovalent vesicular stomatitis virus-based vaccine protects nonhuman primates against heterologous challenge with Bundibugyo ebolavirus. *J Infect Dis*. 2011;204(suppl 3):S1082–S1089. (17) Geisbert TW, Geisbert JB, Leung A, et al. Single-injection vaccine protects nonhuman primates against infection with Marburg virus and three species of Ebola virus. *J Virol*. 2009;83:7296–7304.

the secreted proteins $GP_{1,2\Delta}$, 34 sGP,[211] ssGP,[33] and Δ-peptide.[212,213] Although the function of these molecules is unclear, large concentrations of at least sGP in the serum of infected animals suggest that they might interfere with the host immune response by, for instance, serving as decoys for anti-$GP_{1,2}$ antibodies.[211]

Once inside the cell, several filovirus proteins actively suppress the innate cellular immune response.[214] VP35 protects double-stranded viral RNA intermediates produced during genome replication to prevent recognition by the host pattern recognition receptors melanoma-differentiation-associated protein-5 and retinoic-acid-inducible protein-1. Filovirus VP35 is also a powerful host cell RNA silencing suppressor,[215] and $GP_{1,2}$ antagonizes the cellular viral restriction factor tetherin.[216] In addition, VP35 inhibits interferon response factor 3 and 7 phosphorylation and inhibits interferon (IFN) α/β production.[217–219] Ebolavirus VP24 prevents karyopherin shuttling from the cytoplasm into the nucleus and thus inhibits host cell signaling downstream of IFN-α/β/γ.[220–222] In the case of marburgviruses, VP40—but not VP24—interferes with the IFN pathway. MARV VP40 inhibits signal transducer and activator transcription phosphorylation in response to type I and II IFN and interleukin-6 (IL-6).[223]

The sequence of events during MVD and EVD pathogenesis is likely determined by the accessibility of susceptible cell types for filovirions, the route of infection, and the responses of these cells to infection. Sessile and mobile cells of the mononuclear phagocytic system (alveolar, peritoneal, pleural macrophages; Kupffer cells; microglia) and dendritic cells are initially infected.[20,102,187,224–229] Filoviruses then spread via the lymphatics to regional lymph nodes and via blood to the liver, spleen, and other organs.[224,228,230]

In fundamental ways, macrophages and dendritic cells react differently to filovirus infections. Macrophages are activated upon infection and react with the release of proinflammatory cytokines, such as IL-1β, IL-6, and IL-8, and tumor necrosis factor-α (TNF-α), while releasing progeny virions.[208,227,231–236] This release results in a recruitment of additional macrophages to the infection site, resulting in a vicious cycle of infection of additional macrophages. Increasing amounts of virions are released and spread through the blood, and they are measurable as increased viremia.[224] Dendritic cells, however, react with aberrant responses to infection. Major histocompatibility complex class II is partially suppressed, and expression of tissue factor and the TNF-related apoptosis-inducing ligand is increased.[237,238]

Together, these responses may be the cause of observed death of bystander lymphocytes and general lymphoid hypoplasia in lymph nodes, spleen, and thymus. Lymphoid hypoplasia with the inhibition of IFN pathways at least partially explains the pronounced immunosuppression observed in people with fatal infections.[239,240]

The combined effects of the initial events in filovirus infection probably lead to broad organ system dysregulation, exposure of previously shielded filovirus-susceptible cells, or the transformation of resistant to susceptible cells. Endothelial cells are activated through filovirus infection-induced cytokines.[241] This activation is marked by increased expression and/or release of intravascular adhesion molecule-1, vascular cell adhesion molecule-1, and E- and P-selectin. Consequently, breakdown of the endothelial barrier function results from induced changes in the cadherin/catenin composition of adherens junctions in vascular endothelial cells.[230,234,242–244] The result of this dysregulation is probably massive fluid redistribution (third spacing), which is evidenced clinically by widespread edema and possibly hypovolemic shock. Later in infection, endothelial cells, adrenal cortical cells, epithelial cells, reticular fibroblasts, hepatocytes, and reproductive cells among others also become directly infected with filoviruses, leading to cytolytic infection.[20,187,208,224,245–248]

The increasing concentrations of proinflammatory cytokines (IL-1, IL-6, IL-8, TNF-α, MCP-1, MIP-1α) and other mediators, such as tissue factor, probably form the basis for the induction of disseminated intravascular coagulation (DIC).[249–253] The destruction of adrenal cortical cells results in decreased steroid synthesis, leading to hypotension and therefore further stasis in blood vessels, which may fuel DIC.[208] Consequently, numerous microthrombi form in the vascular system and occlude smaller blood vessels especially. Hypoxemic infarcts develop in downstream tissue, manifested as multiple focal necroses in gonads, kidneys, liver, spleen, and other organs. The continuous destruction of the liver, clinically measurable as an increase in liver enzyme concentrations, leads to a dramatic decrease in albumin and clotting factors. Decreased albumin leads to further fluid redistribution (edema). DIC ceases once all circulating clotting factors have been consumed and leads to petechial rashes, ecchymoses, and general uncontrolled (but rarely life-threatening) hemorrhages.[187,246,254,255] Multiple organ dysfunction syndrome frequently occurs as a result of this series of events.

Clinical Presentation

MVD and EVD cannot be distinguished on grounds of clinical observation alone. Based on the few larger cohort studies published, few to no statistically significant differences were noted in the onset, duration, frequency,

or type of clinical signs and symptoms of MVD and EVD caused by the six filoviruses. Clinical presentation is overall dynamic, with most "typical" signs occurring in many—but not all—cases of infection.[171–177]

The current understanding of human filovirus disease is based largely on observations made during the MVD and EVD outbreaks resulting from MARV infection in Frankfurt/Main and Marburg in West Germany in 1967[71,192,256–259] and in Durba and Watsa in the Democratic Republic of the Congo in 1998–2000[174]; EVD outbreaks resulting from EBOV infection around Yambuku, Zaire, 1976,[260,261] Kikwit, Zaire, 1995,[171] Boene (Democratic Republic of the Congo),[178] and in Western Africa, 2013–2015[172,173,262]; an EVD outbreak resulting from SUDV infection around Yambio, Sudan, 1976[263]; an EVD outbreak resulting from BDBV infection in Bundibugyo, Uganda, 2007[121,175] (Table 23-5); and single-case exportations or laboratory accidents. The incubation period of filovirus disease is highly variable (3–25 days, probably dependent on the route of infection and the amount of virus transmitted). Phase 1, the prodromic phase of the disease that coincides with virema, lasts 5 to 7 days and generally resembles influenza. This phase is characterized by a sudden onset of fever (>38.6°C) and chills, abdominal pain, arthralgia, cough, chest pain and shortness of breath, severe headaches, myalgia, pharyngitis, and the appearance of a morbilliform/maculopapular rash (which, however, may be difficult to see on black skin).

Phase 2 begins after 1 to 2 days of relative remission with a more dramatic clinical presentation including almost all organ systems. Severe abdominal pain, vomiting, and watery diarrhea mark the gastrointestinal effects of infection. Confusion, tremors, psychosis, and coma demonstrate the involvement of the central nervous system. Edema and orthostatic hypotension are both primary and secondary effects of filovirus replication in a variety of cells in the liver, adrenal glands, and vascular system. Induced DIC followed by a total lack of coagulative responses lead to hemorrhagic manifestations. Hemorrhage, which appears in only about 50% of the cases, includes bleeding from mucosal surfaces (gums, nose, rectum, vagina) and venipuncture sites, resulting in detectable blood in sputum, feces, urine, and vomit. Other hemorrhagic manifestations are subconjunctival hemorrhage, petechiae, purpura, and ecchymoses.[171,191,233,238,251,256,257,264–267] On palpation, hepatomegaly is usually prominent, but jaundice is typically absent. Given the filovirus-induced immunosuppression, secondary bacterial and/or fungal infections may develop.

Death is usually the result of multiple organ dysfunction syndrome. Although multiple organ dysfunction syndrome may be caused by fluid redistribution (third spacing) and the multiple necroses in organs, death resulting from blood loss is extremely rare and occurs most frequently among women in labor.[268–270] Survival of EVD, which is inversely correlated with viremia, is associated with particular immunoglobulin M and immunoglobulin G responses and a strong proinflammatory response early in the course of disease,[249,250,252,271–273] and it is probably influenced by host genetics.[274] Survivors of MVD and EVD may experience a wide variety of long-term sequelae that include amnesia, anxiety, joint pain, skin peeling and hair loss, fatigue, hepatitis, myalgia, myelitis, ocular manifestations (choroiditis, iridocyclitis, iritis, uveitis), hearing loss, orchitis, and/or psychosis. At least three filoviruses, MARV, EBOV, and SUDV, may induce persistent infections in the liver, eye, or gonads beyond reconvalescence, and may later reactivate or be transmitted sexually.[120,275–280]

Typical clinical laboratory parameters of MVD and EVD are progressing leukopenia (as low as 1,000/μl) caused by the loss of lymphocytes with a left shift followed by leukocytosis resulting from an increase in granulocytes, and mild thrombocytopenia (50,000–100,000/μl). Liver, kidney, and pancreas dysfunction are evident in the form of increased concentrations of aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, amylase, creatinine, and urea, as well as hypokalemia. The effect of filovirus infection on the coagulation cascade becomes evident via prolonged prothrombin time and partial thromboplastin time and increased D-dimer concentrations.[84,171–173,189,191,257,261,281–283]

DIAGNOSIS

MVD or EVD should be considered in any acutely febrile patient who resides or has travelled through a filovirus-endemic area. A history of rural travel, expeditions into the rain forest or natural or artificial caves, and contact with sick or deceased animals, including humans, should raise suspicion. However, as the recent 2013–2015 EVD outbreak in Western Africa demonstrated, filoviruses may be more broadly distributed than previously thought. Thus, the possibility of filovirus infection in a febrile patient from an African country without recorded filovirus infection should not be discounted. Unfortunately, MVD and EVD patients present with rather unspecific, influenza-like clinical signs caused by numerous pathogens that are more commonly encountered. Even later stages of MVD and EVD are easily confused with the clinical presentation of other diseases (Table 23-6).

TABLE 23-5

CLINICAL PRESENTATION OF MARBURG VIRUS DISEASE OR EBOLA VIRUS DISEASE (ADAPTED AND AVERAGED FROM DATA SOURCES)

Clinical Signs and Symptoms in Humans	Survivors of MARV Infection (%)	Fatal MARV Infections (%)	Survivors of BDBV Infection (%)	Fatal BDBV Infections (%)	Survivors of EBOV Infection (%)	Fatal EBOV Infections (%)
Abdominal pain	59	57	73	88	27	26
Anorexia/appetite loss	77	72	68	77		
Anuria			13	18		
Arthralgia or myalgia	55	55	74	80	25	51
Asthenia			73	82	13	61
Bleeding from the gums	23	36		9		
Bleeding from any site	59	71	29	54	22	47
Bleeding from GI tract				6	19	
Chest pain	18	4	13	45		
Confusion/disorientation			27	36		19
Conjunctival injection/ conjunctivitis	14	42	47	55	13	
Cough	9	5	7	36	13	17
Diarrhea	59	56	83	92	27	44
Difficulty breathing/ distress	36	58	18	88	8	23
Dizziness					13	56
Epistaxis	18	34	7	9		
Facial/neck edema		92		82		24
Fever	10	29	78	81	71	74
Headaches	73	79	82	85	57	46
Hematemesis	68	76		18		
Hemoptysis	9	4		9		
Hiccups	18	44	18	40	2	13
Lumbar pain	5	8	5	36		
Maculopapular rash/ rash			25	27		
Malaise or fatigue	86	83	56	100		
Melena	41	58		27		
Nausea and vomiting	77	76	76	88	59	62
Petechiae	9	7				3
Sore throat, odynophagia, or dysphagia	43	43	45	50	13	31

See Figure 23-1 for color explanations.
BDBV: Bundibugyo virus
EBOV: Ebola virus
GI: gastrointestinal
MARV: Marburg virus
Data sources: (1) 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. (2) Bah EI, Lamah MC, Fletcher T, et al. Clinical presentation of patients with Ebola virus disease in Conakry, Guinea. *N Engl J Med*. 2015;372:40–47. (3) Schieffelin JS, Shaffer JG, Goba A, et al. Clinical illness and outcomes in patients with Ebola in Sierra Leone. *N Engl J Med*. 2014;371:2092–2100. (4) Bausch DG, Nichol ST, Muyembe-Tamfum JJ, et al. Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *N Engl J Med*. 2006;355:909–919. (5) MacNeil A, Farnon EC, Wamala J, et al. Proportion of deaths and clinical features in Bundibugyo Ebola virus infection, Uganda. *Emerg Infect Dis*. 2010;16:1969–1972. (6) Roddy P, Howard N, Van Kerkhove MD, et al. Clinical manifestations and case management of Ebola haemorrhagic fever caused by a newly identified virus strain, Bundibugyo, Uganda, 2007–2008. *PLoS One*. 2012;7:e52986. (7) Barry M, Traore FA, Sako FB, et al. Ebola outbreak in Conakry, Guinea: epidemiological, clinical, and outcome features. *Med Mal Infect*. 2014;44:491–494.

Once filovirus infection is suspected, it is imperative to contact the proper public health authorities and infectious disease specialists and to perform all patient contact and sample handling with utmost caution.[284] Filovirus infection can be confirmed safely and relatively easily in mobile field laboratories, local hospitals, and/or reference laboratories as long as the necessary technology and trained staff are available. Reverse

TABLE 23-6

MARBURG VIRUS DISEASE AND EBOLA VIRUS DISEASE DIFFERENTIAL DIAGNOSIS (ADAPTED FROM DATA SOURCES)

Viral Infections	Bacterial Infections	Fungal Infections	Parasite Infections	Noninfectious Diseases
Major: fulminant viral hepatitides; measles; rubella; VHFs caused by Lassa virus or yellow fever virus	Major: Enterohemorrhagic *Escherichia coli* enteritis; gram-negative bacterial septicemia; leptospirosis; murine typhus; rickettsial diseases; shigellosis; typhoid fever; typhus	Major: histoplasmosis	Major: falciparum malaria	Major: acute promyelocytic leukemia; factor VII, IX, and X deficiencies; hemolytic uremic syndrome; hereditary hemorrhagic telangiectasia; Kawasaki disease; platelet and vascular disorders; snake envenomation; thrombotic thrombocytopenic purpura; warfarin intoxication
Minor: chikungunya, hepatitis A, B, non-A/B; herpes simplex; influenza; mononucleosis; Sindbis disease; West Nile virus fever; VHFs caused by other viruses	Minor: anthrax; bartonellosis; campylobacteriosis; meningococcal septicemia; plague; *Pseudomonas* infections; psittacosis with endocarditis; Q fever; relapsing fever; staphylococcal septicemia; streptococcal septicemia/rheumatic fever	Minor: candidiasis	Minor: trypanosomiasis, visceral leishmaniasis	Minor: drug rashes

VHF: viral hemorrhagic fever

Data sources: (1) Kuhn JH. *Filoviruses. A Compendium of 40 Years of Epidemiological, Clinical, and Laboratory Studies.* In: Carlisher CH, ed. *Archives of Virology* Supplementa Series, Vol 20. Vienna, Austria: Springer-Verlag Wien; 2008. (2) 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. (3) Boisen ML, Schieffelin JS, Goba A, et al. Multiple circulating infections can mimic the early stages of viral hemorrhagic fevers and possible human exposure to filoviruses in Sierra Leone prior to the 2014 outbreak. *Viral Immunol*. 2015;28:19–31.

transcriptase-PCR is the method of choice for detection of filovirus genomes (detection limit: ≈1,000–2000 genome copies/ml of serum) in, for instance, guanidinium isothiocyanate-inactivated samples.[285–287] The less sensitive antigen-capture ELISA and antibody-capture ELISA are alternative or complementary assays for the detection of filovirus proteins and anti-filovirus antibodies in 60Co-irradiated samples.[288,289] Samples from skin biopsies can be inactivated by formalin fixation and then used to diagnose filovirus infection using immunohistochemistry or in situ hybridization.[102,290]

Noninactivated samples, such as acute-phase serum or blood which typically contain high filovirus titers and antifilovirus antibodies,[291] must not be handled outside of a maximum-containment (biosafety level 4) laboratory. Such samples should be collected with utmost caution using proper PPE and then sent to the appropriate World Health Organization reference laboratories using suitable transport media. Filoviruses typically grow quickly and to high titers in standard cell cultures such as grivet Vero E6, rhesus monkey MA-104, or human adrenal carcinoma SW-133 cells.[292,293] From these infected cells, additional studies can be performed, such as variant isolation and typing, sample virus quantification by plaque assays, standard (consensus) Sanger genome sequencing, and easy visualization of typical shapes of filovirions using electron microscopy.[294,295] An overview of current diagnostic options is provided in Table 23-7.

TABLE 23-7

LABORATORY DIAGNOSIS OF FILOVIRUS INFECTION (ADAPTED FROM DATA SOURCES)

PRIMARY ASSAYS

Diagnostic Test	Target	Clinical Material	Advantage	Disadvantage
RT-PCR	Filovirus subgenomic, genomic, or antigenomic nucleic acids	Blood, serum, tissue	Rapid; ultra-sensitive; specific; can be performed on inactivated samples	Requires PCR machine; laboratory cross-contamination can lead to false-positive results; release of RT-PCR inhibitors from tissue can lead to false negative results
Antigen-capture ELISA	Filovirus antigen/ proteins	Blood, serum, tissue possible	Rapid; sensitive; specific; can be performed on inactivated samples; high-throughput	Requires ELISA reader
IgG-capture ELISA	Antifilovirus antibodies (late in infection; survivors)	Serum	Rapid; sensitive; specific; can be performed on inactivated samples	Requires ELISA reader and large amounts of purified native or recombinant filoviral antigen; some patients do not seroconvert
IgM-capture ELISA	Antifilovirus antibodies (early in infection)	Serum	Rapid; sensitive; specific; can be performed on inactivated samples	Requires ELISA reader and large amounts of purified native or recombinant filoviral antigen; some patients do not seroconvert

SECONDARY/CONFIRMATORY ASSAYS

Diagnostic Test	Target	Source	Advantage	Disadvantage
Virus isolation	Filoviruses	Blood, tissue	Specific	Requires maximum-containment laboratory and time; filovirus isolation may fail or filovirus replication may not cause CPE in cell cultures during initial passages
Electron microscopy	Complete or fragmented filovirions or characteristic cellular inclusion bodies	Blood, serum, tissue	Specific	Insensitive; requires electron microscope
Indirect immuno-fluorescent assay	Antifilovirus antibodies	Serum	Rapid; simple; safe	Insensitive; possible cross reactions leading to false positive results; subjective interpretation; some patients do not seroconvert
Fluorescent assay	Filovirus antigen/ proteins	Tissue culture/ isolated virus	Rapid; simple; safe	Insensitive; requires infectious material and specific antibodies; subjective interpretation
Next-generation sequencing	Filovirus subgenomic, genomic, or antigenomic nucleic acids	Blood, serum, tissue	Very specific; ultra-sensitive; can determine coding-complete filovirus genomes in absence of virus culture; allows molecular epidemiology	New and expensive technology; not yet widespread; requires highly trained personnel and bioinformatics support
Immunohisto-chemistry	Filoviral antigen	Tissue (skin, liver)	Tissue can be fixed	Requires time and specific antibodies

(**Table 23-7** *continues*)

Table 23-7 *continued*

In situ hybridization	Filoviral nucleic acids	Tissue	Tissue can be fixed	Requires special equipment and specific probes
Western blot	Antifilovirus antibodies	Serum	Specific	Difficult interpretation; requires specific antibodies

CPE: cytopathic effect
ELISA: enzyme-linked immunosorbent assay
IgG: immunoglobulin G
IgM: immunoglobulin M
RT-PCR: reverse transcriptase polymerase chain reaction
Data sources: (1) Kuhn JH. *Filoviruses: A Compendium of 40 Years of Epidemiological, Clinical, and Laboratory Studies*. In: Calisher CH, ed. *Archives of Virology* Supplementa Series, Vol 20. Vienna, Austria: Springer-Verlag Wien; 2008. (2) Gire SK, Goba A, Andersen KG, et al. Genomic surveillance elucidates Ebola virus origin and transmission during the 2014 outbreak. *Science*. 2014;345:1369–1372. (3) 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. (4) Wang YP, Zhang XE, Wei HP. Laboratory detection and diagnosis of filoviruses. *Virol Sin*. 2011;26:73–80.

TREATMENT

Treatment of MVD and EVD patients is challenging because of the healthcare workers' risk of infection. Patients should be isolated, infection control precautions/strict barrier-nursing techniques need to be implemented, and healthcare personnel must wear proper PPE. In addition, standard operating procedures should be in place for safe clinical sample management; decontamination of possibly contaminated tools, materials or surface equipment and personnel; point-of-care laboratory testing; and infectious waste management.

No FDA-approved specific therapy is available to treat human infections. Although statistical reports are still lacking, chances for survival from MVD and EVD are now thought to be dramatically increased through aggressive supportive therapy.[262,296,297] Treatment should follow the guidelines for severe sepsis management and therefore aim to reestablish fluid and electrolyte balance and reversal of DIC, hemorrhage, hypotension/hypoperfusion, acute kidney injury, and shock. As filoviruses potently suppress the immune system, empirical and possibly prophylactic treatment of secondary bacterial and/or fungal infections with broad-spectrum antibiotics (eg, vancomycin, piperacillin/tazobactam) and antimycotics is advised. Pain management and administration of antiemetics should always be considered.[180,181,262]

Numerous drugs have been evaluated in vitro and in various animal models over the years to identify candidate MCMs to treat MVD and EVD.[298–302] Although none of them has reached medical licensure, several have been sufficiently promising for emergency use in humans. An overview of the most commonly discussed MCMs for filovirus disease treatment is provided in Table 23-8.

SUMMARY

Due to extremely low human case numbers, filovirus infections were long considered exotic infectious diseases of no larger consequence to global public health. The still ongoing EVD outbreak in Western Africa, which by now includes close to 28,500 cases and 11,300 deaths, brought awareness to the fact that single filovirus introductions into the human population may lead to devastating and large epidemics that can spread quickly across international borders. Unfortunately, despite considerable scientific progress, many key questions regarding filoviruses remain to be answered. First, and foremost, the natural filovirus host reservoirs have to be identified so that preventive measures against initial zoonotic spillover into humans can be established. Second, almost all filovirus research currently focuses only on EBOV, SUDV, and MARV, while next to nothing is known about the molecular or pathogenic features of other filoviruses. Yet, medical countermeasures should be created that can be used against any human (and possibly) animal infection because it is currently unpredictable which filovirus will strike next.

TABLE 23-8

SELECTED PROMISING CANDIDATE THERAPEUTICS FOR FILOVIRUS INFECTIONS

Candidate MCM	Mechanism of Action	Efficacy	Additional Information
ZMapp (Mapp Biopharmaceutical, Inc/LeafBio, Inc)[1]	Cocktail of three monoclonal antibodies (c13C6, c2G4, c4G7) targeting **EBOV** $GP_{1,2}$	100% survival of rhesus monkeys up to 5 days after **EBOV** exposure	Produced in genetically modified tobacco plants; may not be scalable; requires frozen shipping. Currently in phase 1/2 clinical trials
MB-003 (USAMRIID/ Mapp Biopharmaceutical)[2,3]	Cocktail of three monoclonal antibodies (c13C6, h-13F6, c6D8) targeting **EBOV** $GP_{1,2}$	100% survival of rhesus monkeys 1 h after **EBOV** exposure; 67% and 43% survival at 2 days and 4–5 days after exposure, respectively	Produced in genetically modified tobacco plants; scalable; requires frozen shipping
ZMAb (PHAC/Defyrus Inc)[4–7]	Cocktail of three monoclonal antibodies (1H3, 2G4, 4G7) targeting **EBOV** $GP_{1,2}$	100% survival of crab-eating macaques 1 day after **EBOV** exposure; 50% survival at 2 days after exposure. 100% survival of survivors after reexposure to **EBOV** 10 weeks after challenge, 67% survival after 13 weeks	Requires frozen shipping
Immucillin-A/BCX4430 (BioCryst Pharmaceuticals)[8]	Nucleoside analog that inhibits the **MARV** RNA-dependent RNA polymerase and causes lethal mutagenesis	100% survival of crab-eating macaques 48 h after **MARV** exposure	In phase 1 clinical trial
Favipiravir/T-705 (Fujifilm/Toyama Chemical Co, Ltd)[9,10]	Nucleotide analog that inhibits the filovirus RNA-dependent RNA polymerase and causes lethal mutagenesis	100% survival of IFNAR-/- laboratory mice 6 days after parenteral mouse-adapted **EBOV** exposure; 17% survival of rhesus macaques	Used as a licensed antiinfluenza drug in Japan. Contraindicated in pregnancy because of possibility of teratogenicity and embryotoxicity. In phase 3 clinical trials (FLUAV). Currently is being evaluated on EVD patients in Guinea in a single-arm phase 2 clinical trial
JK-05 (Sihuan Pharmaceutical Holdings Group, Ltd and Academy of Military Medical Sciences)[11]	Nucleotide analog that inhibits the filovirus RNA-dependent RNA polymerase and causes lethal mutagenesis	Efficacy in laboratory mice	Considered for use in emergency situations
TKM-Ebola/Tekmira-100802 (Tekmira Pharmaceuticals Corp)[12,13]	Lipid nanoparticle cocktail of siRNAs targeting **EBOV** VP35, VP24, and L	100% survival of rhesus monkeys 30–60 min after **EBOV** exposure; 83%, 50%, and 67% survival at 1, 2, and 3 days after exposure, respectively	Phase 1 clinical trial aborted
"TKM-Marburg" (Tekmira Pharmaceuticals Corp)[14]	Lipid nanoparticle cocktail of siRNAs targeting **MARV** NP	100% survival of rhesus monkeys 30–45 min, 1 day, 2 days, and 3 days after **MARV** exposure	
AVI-7537 (Sarepta Therapeutics)[15]	Phosphorodiamidate morpholino oligomer targeting **EBOV** VP24	63% survival of rhesus monkeys 1 h after **EBOV** exposure	Phase 1 clinical trial

(**Table 23-8** *continues*)

Table 23-8 *continued*

AVI-6002 (Sarepta Therapeutics)[16]	Phosphorodiamidate morpholino oligomer targeting **EBOV** VP35 and VP24	>60% survival of rhesus monkeys 30–60 min after **EBOV** exposure	Phase 1 clinical trial completed
rVS[I]VΔG-ZEBOV-GP/ BPSC1001 (Newlink Genetics/PHAC)[17]	Postexposure vaccine consisting of a recombinant replicating vesicular stomatitis Indiana virus expressing **EBOV** $GP_{1,2}$ to stimulate anti-$GP_{1,2}$ immune responses	50% survival of rhesus monkeys 20–30 min after **EBOV** exposure; 100% and 50% survival of laboratory mice and guinea pigs, respectively, 24 h after **EBOV** exposure	Easy to produce; requires frozen shipping; concerns about immunocompromised patients. Currently in phase 1 clinical trials
Other rVS[I]V formulations[18–20]	Postexposure vaccine consisting of a recombinant replicating vesicular stomatitis Indiana virus expressing filovirus $GP_{1,2}$ to stimulate anti-$GP_{1,2}$ immune responses	**MARV**: 100% survival of rhesus monkeys 20–30 min after **MARV** exposure; 83% and 33% survival at 1 day and 2 days after exposure, respectively SUDV: 100% survival of rhesus monkeys 20–30 min after **SUDV** exposure	Easy to produce; requires frozen shipping; concerns about immunocompromised patients
Recombinant Nematode Anticoagulant Protein c2 (rNAPc2)[21]	Inhibits factor VIIa/tissue factor complex and blood clot formation	33% survival of rhesus monkeys 10 min and 1 day after **EBOV** exposure	In phase 2 clinical trial for second-line treatment of metastatic colorectal carcinoma in combination with contemporary 5-FU-based chemotherapy
Activated drotrecogin alfa/Xigris (Eli Lilly and Company)[22]	Recombinant human activated protein C; inhibits coagulation factors Va and VIIIa (antithrombotic)	20% survival of rhesus monkeys 1 h after **EBOV** exposure	Withdrawn from market
Hyperimmune equine[23,24] immunoglobulin G[23–25]	Filovirions	50%–100% survival of hamadryas baboons 5–15 min after **EBOV** exposure; 80%, 20%–100%, and 29% survival at 30 min, 1 h, and 2 h after exposure, respectively	Licensed in Russia for treatment of occupational accidents. Highly immunogenic in humans. Evaluation in rhesus monkeys (using different dose and virus variant) not successful
Passive transfer of convalescent or postimmunization plasma[26–28]	Filovirions	Passive transfer of concentrated polyclonal IgG from immune rhesus monkeys resulted in 100% survival of rhesus monkeys at 15–30 min and 48 h after **MARV** exposure	Uncontrolled experiment during the 1995 EVD/**EBOV** outbreak in Kikwit, Zaire, suggested passive transfer of whole blood to be protective for 7 of 8 patients

See Figure 23-1 for color explanations.
ADE: adverse effects
ADV: adenovirus
CMV: cytomegalovirus
EBOV: Ebola virus
EVD: Ebola virus disease
FLUAV: influenza A virus
5-FU: fluorouracil
GP: glycoprotein
IFN: interferon
IFNAR: interferon-α/β receptor
IgG: immunoglobulin G
MARV: Marburg virus
MCM: medical countermeasures
PHAC: Public Health Agency of Canada
siRNA: short interfering RNA
SUDV: Sudan virus
USAMRIID: US Army Medical Research Institute of Infectious Diseases
VP: viral protein

(**Table 23-8** *continues*)

Table 23-8 *continued*

Note: Information on the status of all ongoing filovirus-relevant clinical trials can be found at https://ClinicalTrials.gov with the search terms "Ebola" or "Marburg."

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