Filoviridae: a Taxonomic Home for Marburg and Ebola Viruses ?

M.P.		Kiley ^a
E.T.W.		Bowen ^b
G.A.		Eddy ^c
M.		Isaäcson ^d
K.M.		Johnson ^a
J.B.		McCormick ^a
F.A.		Murphy ^c
S.R.		Pattyn ^f
D.		Peters ^g
O.W.		Prozesky ^h
R.L.		Regnery ^a
D.I.H.		Simpson ^b
W.		Slenczka ⁱ
P.		Sureau ^j
G.		van der Groen ^f
P.A.		Webb ^a
H.		Wulff ^a

^aCenter for Infectious Diseases, Centers for Disease Control, Atlanta, Ga., USA; ^bPHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, UK; ^cUS Army Medical Research Institute for Infectious Diseases, Ft. Detrick, Frederick, Md., USA; ^dThe South African Institute for Medical Research, Johannesburg, South Africa; ^eColorado State University, College of Veterinary Medicine and Biomedical Sciences, Ft. Collins, Col., USA; ^fPrins Leopold Instituut voor Tropische Geneeskunde, Antwerpen, Belgium; ^gBernard-Nocht-Institut für Schiffs- und Tropenkrankheiten, Abteilung für Virologie, Hamburg, FRG; ^hNational Institute for Virology, Sandringham, Transvaal, South Africa; ⁱHygiene-Institut der Universität, Marburg, FRG; ^jInstitut Pasteur, Paris, France

Address inquiries to: Dr. M. P. Kiley, Special Pathogens Branch, Viral Diseases Division, Center for Infectious Diseases, Centers for Disease Control, Atlanta, GA 30333 (USA)

Marburg (MBG) and Ebola (EBO) are viruses indigenous to Africa which cause hemor-rhagic fever in man [1,2]. They share a unique morphology but show only minimal or no antigenic cross-reactivity [3]. They are extremely virulent viruses designated as class 4 (biosafety level 4) viral pathogens [4] for which maximum biological containment (P-4) has been recommended. For these reasons, fundamental data necessary for taxonomic purposes have only now begun to emerge.

Attempts to study the taxonomy of these viruses were made soon after the isolation of MBG virus. Several early proposals were based on virion morphology and limited physico-chemical data. One proposal, which is still favored by some, suggested that MBG virus (and presumably the more recently discovered EBO virus) be included in the rhabdovirus group [5]. The term ‘torovirus’ was suggested by *Almeida et al.* [6] because they believed that the mature form of the virus was a doughnut-shaped structure, or a ‘torus’ in mathematical terminology. Soon after the isolation of EBO virus in 1976, another name for these viruses was proposed. Since the predominant forms of these viruses most frequently seen in the electron microscope in negatively stained preparations are long tubular structures, the name ‘tuburnaviruses’ was suggested by *Simpson and Zuckerman* [7]. None of these proposals was formally submitted to the International Committee on Taxonomy of Viruses (ICTV). The issue of virus nomenclature was raised again at a symposium on EBO virus disease held at Antwerp in 1977 and at a meeting of the ICTV Executive Committee. The rhabdovirus subcommittee chaired by Dr. *Fred Brown* was as-

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signed responsibility for making a proposal to ICTV and, after circulation of available data to members, decided that available information was insufficient to reach a decision. Additional properties of MBG and EBO have now been described. The present taxon-omic proposal resulted from discussions concerning this new information held during a ‘Congress on Virology and Class-4 Agents’ in Johannesburg in late 1979. We here describe the known characteristics of these viruses, which we believe warrant family status, and suggest the name ‘Filoviridae’ (filo, Latin ‘filament, thread’) for this taxon. The major features that distinguish these viruses from the rhabdoviruses, their morphologically close neighbors, are: (i) particle length, (ii) unique proteins, and (iii) a central axial channel of diameter significantly smaller than that of the rhabdoviruses. Other names considered were Nemaviridae (nema, Greek ‘filament’), Fibra-viridae (fibra, Latin ‘fiber, filament’), Funi-viridae (from Latin *funis*, ‘rope, cord’), and Virgaviridae (virga, Latin ‘streak, cane’).

The general features of MBG and EBO viruses are as follows:

A. Properties of the Virus Particle Nucleic acid: The virion contains one molecule of single-stranded RNA with a molecular weight (MW) of approximately 4.2×10^6 . Virion RNA is not infectious, does not bind to oligo-dT-cellulose, and is therefore thought to be a negative-sense strand [8; *Regnery et al.*, unpublished].

Protein: Purified virions contain at least 5 polypeptides with the basic pattern being the same for both viruses. The proteins are designated VPO, VPI, VP2, VP3, and VP4 with corresponding EBO MWs of $\approx 190,000$, 125,000, 104,000, 40,000, and 26,000. MBG virions contain similar proteins with MWs of $\approx 190,000$, 140,000, 98,000, 38,000, and 22,000. In both viruses, VPI is a glycoprotein and probably is the major component of the virion spikes since it is removed by bromelain treatment. Both VP2 and VP3 are associated with the 1.32 g/cm^3 viral ribonucleoprotein (RNP) obtained by detergent-high salt treatment of virions [9; *Kiley*, unpublished].

Lipid: Lipid solvents destroy viral infectivity [10–12] and release a 1.32 g/cm^3 RNP from the virions [9]. The percentage of virion lipid is unknown.

Carbohydrate: Sugar is a component of one virion protein [9] and possibly glycolipid.

Physicochemical properties: The virion has a MW of approximately $(300-600) \times 10^6$. Larger particles have a very high sedimentation coefficient, and that of a uniform bacilliform particle is approximately 1400S [Regnery et al., unpublished]. Density is 1.14 g/cm^3 in potassium tartrate [9]. Infectivity is quite stable at room temperature but is destroyed in 30 min at 60° [10]. Virus is inactivated by UV and gamma irradiation [13a, 13b] and by 1% formalin [10] and β -propiolactone [12]. Both viruses are inactivated by brief exposure to commercial phenolic disinfectants [Lange et al., unpublished] and are very sensitive to lipid solvents [10-12].

Morphology: The virions of EBO and MBG are very similar in morphology, and the following general morphological description is derived from several sources [5, 14-16]. By electron microscopy, MBG and EBO particles are pleomorphic, appearing as long filamentous forms (sometimes with extensive branching) or as U-shaped, '6'-shaped, or circular forms. The particles vary greatly in length (up to 14,000 nm), but have a uniform diameter of approximately 80 nm. There are spikes on the particle surface approximately 70 Å in length and 100 Å from one another.

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Beneath the virion envelope lies a complex nucleocapsid structure consisting of a dark, central axis of 20 nm diameter surrounded by a helical tubular capsid of approximately 50 nm bearing cross-striation with a periodicity of about 5 nm. Because its diameter is that of the tubular structures found in intracellular inclusions, the 20-nm dark central axis is presumed to be the virion RNP [Peters, unpublished]. This probably corresponds to the 1.32 g/cm^3 structure released from virions by detergent treatment and consisting of virion RNA and the VP2 and VP3 virion proteins [9]. Within the nucleocapsid is an axial channel of approximately 10-15 nm. While the lengths of MBG and EBO particles vary over a wide range, a recent study with rate-zonal, sucrose-gradient-purified particles indicates that the unit length associated with peak infectivity for MBG is 790 nm and that of EBO is 970 nm [Regnery et al., unpublished]. These results support the previously published work of Peters and colleagues [5,15] who found, in unpurified preparations, a median length of 665 nm for MBG and 805 nm for EBO. In both studies, EBO virions were approximately 1.2 times as long as MBG virions. Also, Peters has found a significant peak of double the median length in both MBG and EBO preparations [15].

Antigenic properties: Details concerning the specific antigenic composition of MBG and EBO are not available. Nevertheless, information has been obtained regarding the antigenic properties of these viruses. Casals [17], using the complement-fixation (CF) test, determined that MBG virus is not serologically related to a variety of arboviruses and rhabdo-viruses.

Immunofluorescent techniques, which have been found to be faster and more sensitive than the CF test [18], demonstrate that there is little

or no antigenic cross-reaction between MBG and EBO viruses [3, 19]. The lack of antigenic relatedness is also indicated by the fact that previous immunity to MBG virus in laboratory animals does not protect against subsequent infection with EBO virus [7].

Immunofluorescent and radioimmune precipitation techniques [3; Richman et al., unpublished], cross-protection studies [20], and Ti oligonucleotide mapping studies [Cox et al., unpublished] also indicate that while the Sudan and Zaire strains of EBO virus share many antigens, they also demonstrate significant antigenic differences.

B. Replication

The morphogenesis of MBG and EBO viruses has been examined in several laboratories [5,11,12,14,15,19,21–23]. Whether investigators studied human liver, guinea pig organs, monkey organs, or tissue culture, a typical pattern emerged. Virions are constructed from preformed nucleocapsids and envelopes which are added by budding through cellular membranes. Virus-infected cells contain prominent cyto-plasmic inclusion bodies consisting of viral nucleoprotein material. These inclusion bodies are complex and distinct, consisting of a finely fibrillar or granular ground substance which condenses into tubular structures, or nucleocapsids. As infection proceeds, these inclusion bodies grow and become highly structured even at sites remote from the cell membranes. Budding of completed virions takes place at cell membranes into which virion spikes have been inserted. It appears that nucleocapsids, at the time of budding, may orient in any plane from perpendicular to parallel to cell membranes, which may produce the branching seen with these viruses [22].

Little is known concerning the macromole-

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cular events accompanying replication of these viruses.

C. Biological Aspects

Ecology: Both viruses are indigenous to Africa and cause hemorrhagic fever in man. MBG was discovered in 1967 when the disease was transmitted to German and Yugoslavian scientists who had come in contact with blood or organs from monkeys imported from Uganda [24,25].

Subsequently, 1 primary and 2 contact cases were reported in 1975 from South Africa [26]. In January 1980, 2 cases occurred in western Kenya [27].

EBO virus was first isolated from two separate outbreaks in northern Zaire and southern Sudan in the fall of 1976 [19,28,29]. Another case occurred in Tandala, Zaire, in 1977 [30], and an additional outbreak occurred in the Sudan in 1979 [31].

Epidemiology: The monkeys implicated as the source of the 1967 MBG outbreak in Germany and Yugoslavia were vervets (*Cerco-pithecus aethiops*) recently imported from Uganda [32]. These monkeys did not appear to be ill, although the species exhibits a severe form of disease when infected experimentally. The infection was transmitted to man only by direct contact with monkey organs or blood (although there is one report of monkey-to-monkey aerosol transmission in the laboratory [33]). Secondary cases occurred either by inadvertent inoculation or in nurses who had direct contact with MBG patients or their blood. One secondary case was in a woman believed to have been infected during sexual intercourse 12 weeks after clinical recovery of her husband [34]. The mortality rate in this outbreak was 23%. 3 cases of MBG disease were reported from South Africa in 1975 [26]. The index case was a tourist who subsequently infected his companion. A tertiary case occurred

in a nurse who attended both patients. In this outbreak, it is possible that the index case may have been infected by the bite of an insect [35]. 2 cases of MBG disease occurred in Kenya in 1980 [27]. The source of the infection is unknown.

EBO virus first appeared as simultaneous outbreaks in northern Zaire and southern Sudan during the summer and fall of 1976 [2, 36,37]. The sources of infection for the index cases are unknown, and transmission was due to either close personal contact with patients or use of contaminated needles. The mortality rate in the Zaire outbreak was 88%, as compared to 53% in Sudan. Another case of EBO hemorrhagic fever occurred in Zaire in 1977 [30] and an outbreak involving 34 cases occurred in the Sudan in the summer of 1979 [31].

Although the Zaire and Sudan EBO isolates are closely related antigenically, they may differ genetically. There may, in fact, be two biotypes or immunotypes of EBO [Cox et al., unpublished; McCormick et al., unpublished].

As is the case with MBG, the ultimate source of EBO virus in nature is unknown.

Host range: The natural reservoir is unknown. In the laboratory, monkey, mouse, guinea pig, and hamster have been experimentally infected [1].

Transmission: In human cases, transmission appears to occur only by close personal contact with a patient or by exposure to infected body fluids or organs [1,2,36,37].

Pathogenesis: The following description of EBO-MBG pathogenesis is derived from studies conducted in a number of laboratories [1,2, 11,12,19–21,23,28,33,38,39].

Both viruses are highly virulent for humans and several species of monkeys. Strains of EBO isolated from Zaire and Sudan differ in virulence for monkeys and mice, the former causing uniformly lethal infection and the latter

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rarely resulting in fatality. MBG and EBO cause fatal disease in guinea pigs and hamsters after appropriate serial transfer in these animals. The incubation period in humans and monkeys is from 4 to 16 days, during which time virus replicates in lymph nodes, spleen, and probably in fixed-tissue macrophages of various organs. The period of acute febrile illness is marked by high viremia and the presence of virus in many viscera. The associated pathological changes are demonstrated by generally severe focal necrosis of hepatocytes with remarkably little inflammatory response and virtually complete sparing of biliary architecture and function. Thus, there is severe chemical hepatitis without chemical or clinical jaundice. Follicular necrosis of lymph nodes and spleen is present, sometimes with hyperplasia of the reticuloendothelial elements of these organs. Scattered hemorrhages are observed later in the course of disease in both humans and monkeys, involve the gastrointestinal tract in particular and, in humans, have been attributed to disseminated intravascular coagulation. Interstitial pneumonia, pancreatitis, orchitis, and iridocyclitis have been described in human infections.

Pathological changes have been attributed to direct viral damage of parenchymal cells. Virions and viral antigens are readily demonstrable in liver and kidney tissues of primates, and antiviral antibodies are rarely detectable before death or resolution of acute clinical disease.

1 Virus group

1.1 Family: Filoviridae (proposed).

Genus: Filovirus.

Type species: Marburg (MBG) virus human.

1.3 Relationships with other groups:

Morphologically similar to rhabdo-

viruses except much longer. Branching may occur, and 6's and U's are seen. Rate-zonal purified virions are long bacilliform rods. Internal helical nucleocapsid.

2 The virion

2.1 Chemical composition

2.1.1 Nucleic acid

RNA

Single-stranded

Linear

Number of pieces: One.

Sedimentation coefficient: 46S (0.15 M NaCl, pH 7.4).

Molecular weight: 4.2×10^6 .

Percentage weight of virion: $\approx 1.1\%$.

Infectivity: RNA not infectious.

Other features: RNA is negative strand, no poly A tract.

2.1.2 Proteins

Percentage weight of virion: Unknown.

Number of polypeptides: At least 5 proteins. Additional polypeptides may be present.

Molecular weights of polypeptides:

MBG EBO

VPO $\sim 190,000$ $\approx 190,000$

VP1 140,000 125,000

VP2 98,000 104,000

VP3 38,000 40,000

VP4 22,000 26,000

2.1.2.4 Number of protein subunits in virion : MBG virions are estimated to contain 39 molecules of L protein, 146 of VP1, 826 of VP2, 6,034 of VP3, and 939 of VP4.

Lipids: Present in membrane.

Carbohydrate

2.1.4.1 Present in glycoprotein and probably glycolipid.

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2.2 Physicochemical properties

Density: 1.14 g/cm^3 in potassium tartrate.

Sedimentation coefficient: Bacilli-form particle = 1,300–1,400S.

2.2.4 Stability of infectivity

Heat: Inactivated at 56° in 60 min.

Lipid solvents: Sensitive to deoxy-cholate and ether (MBG).

Radiation: Inactivated by UV and gamma irradiation ($\approx 10^6$ rad).

Other agents: Sensitive to glutaraldehyde, 2% peracetic acid (EBO), detergents, phenol.

2.3 Structure

2.3.1 Nucleocapsid: RNA-VP2-VP3 complex containing membrane in the virion.

2.3.1.1 Symmetry: Helical.

2.3.1.3 Diameter and number of subunits per turn: Central axis of 20–30 nm surrounded by helically wound capsid of 50–60 nm with a 5-nm periodicity.

2.3.2 Envelope

Dimensions: Thickness approximately 10 nm, closely surrounds nucleocapsid.

Composition: Lipid bilayer containing one glycosylated VP1 and one nonglycosylated VP4.

2.3.3 Cores (RNP)

Dimensions: Tubular outer diameter 23 nm (MBG), 20 nm (EBO); inner diameter (axial channel) 15 nm (MBG), 11.5 nm (EBO), approximately 24 subunits per turn.

Composition: RNP containing two nonglycosylated proteins (VP2, VP3) and 4.2×10^6 MW RNA. RNA is $\approx 5\%$ by weight by density.

2.4 Morphology

Overall shape: Filamentous with branching, folding, and torus formation. Bacilliform rods.

Dimensions: $(130\text{--}14,000) \times \approx 80$ nm. Unit length 790 nm (MBG) and 970 nm (EBO).

Surface projections: 7–10 nm spikes spaced at 10-nm intervals.

2.4.4 Other features: None known.3 Replication

3.1 Site of accumulation of viral proteins : Cytoplasm and plasma membranes.

3.3.2 Effect of inhibitors: Not sensitive to actinomycin D or bromodeoxyuridine.

Site and mechanism of maturation: Nucleocapsid assembled in cytoplasm, virus matures via budding through altered plasma membrane.

Other features: None known.

5 Host range

Natural: Man is apparently incidental host. *Cercopithecus* monkey is an incidental MBG host.

Natural reservoir host not yet identified.

Experimental

In vivo: Monkeys are highly susceptible to infection, with lethal outcome except when infected with EBO strains from Sudan. EBO from Zaire is lethal for suckling mice. Infection can be lethal in guinea pigs, hamsters, and rabbits and is a function of serial passage and viral strain.

In vitro: Both viruses infect a variety of mammalian cell lines.

6 Pathogenicity

6.1 Association with disease: Both viruses produce acute hemorrhagic disease, with significant mortality in man.

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Tissue tropisms: Man, monkey, guinea pig; liver, other visceral organs; lymphoid tissue.

Cytopathology: Variable scruffy destruction of Vero cells. Cytopathic endpoints do not reflect infectivity, which is 10–100 times greater.

Geographic distribution: MBG in Europe (1967) initiated by imported Ugandan monkeys with two small additional outbreaks in South Africa (1975) and Kenya (1980). EBO in Zaire and Sudan (1976, 1979).

Transmission

Vertical: Unknown.

Horizontal: Close, continued contact with patients causes secondary human infection, probably not aerosol-borne.

Vectors: Unknown.

Biological: None reported.

Mechanical: Contaminated needles, semen, blood, and body fluids.

9 Antigenic properties

Number of distinct antigenic specificities in virion: One determined to date by FA.

Antigen(s) involved in virus neutralization : Virus is not neutralizable under conditions tested to date.

Specificity of different antigens: Not known.

Antigenic properties used for classification : Lack of cross-reactivity via immunofluorescence separates MBG from EBO. MBG does not cross-react with a variety of RNA viruses, particularly rhabdoviruses. EBO strains from Zaire and Sudan can be differentiated quantitatively by immunofluorescence and radioimmunoassay and by Ti digests of RNA.

10 Classification

10.1 Brief definition of proposed family: Virions consist of helical nucleocapsid surrounded by unit membrane containing spikes. Unpurified virions are 80 nm in width with a hollow axial channel of < 15 nm and vary from 130 to 14,000 nm in length. Rate-zonal centrifugation shows majority of particles associated with peak infectivity are bacilliforms, 790 (MBG) and 970 (EBO) nm long. Virion contains at least 5 proteins (one glycoprotein) and single-stranded RNA of 4.2×10^6 MW. Cause of hemorrhagic fever with high mortality in man. Geographically restricted to Africa. The two members, MBG and EBO, are not antigenically related.

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