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MARBURG AND EBOLA VIRUSES

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I. Introduction

Filoviruses are among the most pathogenic of human viruses. They are classified as "Biological Level 4" agents (WHO; Risk Group 4) based on their high mortality rate, person-to-person transmission, potential aerosol infectivity, and absence of vaccines and chemotherapy. Maximum containment is required for all laboratory work with infectious material (1, 2). Yet, we are only beginning to understand the interactions of these viruses with their host, and our knowledge on genetics, pathogenicity, and natural history is still limited. Even though outbreaks among human and nonhuman primates to date have always been self-limited, it is because of our ignorance about the natural

reservoir, the potential of these viruses to be transmitted by aerosol, and the lack of immunoprophylactic and chemotherapeutic measures that these infections are of great concern to biomedical scientists. Imported monkeys and international travel, especially rapid travel within the incubation time, are considerable risk factors for introduction of filovirus infections into nonendemic countries. Limited knowledge of the epidemiology and clinical picture of filoviral hemorrhagic fever (HF) and inexperience in diagnosing cases and in case management magnify the danger of an introduction.

Filoviruses, like other RNA viruses, presumably have a potential for rapid evolution due to an inherently high error rate of the virus-encoded polymerase and a lack of repair mechanisms (3). The consequence may be a spectrum of genetic variants that are selected by the host for different transmissibility, virulence, and other biological properties. Changes in socioeconomic structure, such as an increase in human population, increase in speed, variety, and frequency of travel, and disruption of social structures may augment the development of mutant virus populations and the probability of a filovirus emerging as a truly serious public health problem (4).

II. Epidemiology of Filoviruses

A. Outbreaks

1. Marburg Hemorrhagic Fever

Hemorrhagic fever caused by the Marburg virus first emerged in 1967 (Table I). The epidemic started in mid-August with three laboratory workers in a pharmaceutical factory in Marburg, Federal Republic of Germany, who became ill with a hemorrhagic disease after being engaged in processing organs from African green monkeys (*Cercopithecus aelhiops*). In the course of the epidemic 17 more patients were admitted, and two medical staff members became infected while attending the patients in the hospital. In November, the last patient was admitted who apparently had been infected by her husband during the convalescent period (5, 6). Six more cases, including two people with secondary infections, occurred in Frankfurt, Federal Republic of Germany, who apparently developed the disease at the same time (7, 8). Additional cases occurred in September in Belgrade, Yugoslavia: a veterinarian, who became infected while performing an autopsy on dead monkeys, and his wife, who nursed him during the first days of the illness (9). In all, there were 31 cases, including six secondary cases,

and there were seven deaths (Tables I and II) (10). Serologic data obtained some years after the epidemic suggest an additional primary case in Marburg during the 1967 outbreak (W. Slenczka, unpublished data) (Table II). The infective agent was introduced by infected monkeys imported from Uganda, among which a few originally infected animals were probably responsible for the whole episode (Fig. 1). Numbers on hemorrhagic disease and death among the monkeys from the single shipment from Uganda have never been published, but all African green monkeys experimentally inoculated with the virus died (11). Aerosol transmission during the epidemic is very unlikely and infection from monkey to man occurred by direct contact with blood or organs of the animals, including tissue culture (Table II).

Marburg virus (MBG) remained an obscure medical curiosity until 1975, when three cases of Marburg hemorrhagic fever were reported from Johannesburg, South Africa (Table I; Fig. 1) (12). The index patient was a man who had traveled in Zimbabwe shortly before becoming ill. Seven days after onset of his illness, his traveling companion became ill, followed by a nurse who came down with the symptoms 7 days after contact with the second patient. The index case patient died 12 days after onset of the disease, whereas both patients secondarily infected survived. The last two episodes occurred in 1980 and 1987 in Kenya (Table I; Fig. 1). The index patient in 1980 became ill in western Kenya and died in Nairobi. An attending physician became infected but survived. Further spread was prevented, presumably by use of barrier nursing procedures (13). In 1987, a single fatal Marburg case was reported in western Kenya, near where the index patient in the 1980 episode had become infected (E. D. Johnson, unpublished).

2. Ebola Hemorrhagic Fever

Hemorrhagic fever caused by Ebola virus (EBO), another filovirus, emerged in 1976, when two epidemics occurred simultaneously in Zaire and Sudan (Table I). The agent was isolated from patients in both countries and named after a small river in northwestern Zaire. In June and July the first cases were reported from Nzara in western Equatoria Province of southern Sudan, a small town bordering the African rain forest zone (Fig. 1). The outbreak was strongly associated with index cases in a single cotton factory in town, and spread was to close relatives (67 cases). The epidemic was intensified by the spread of cases to neighboring areas, Maridi, Tembura, and Juba. High levels of transmission occurred in the hospital of Maridi, a teaching center for student nurses (213 cases). Despite the similarities of the clinical diseases and mortality rates, the epidemic in Nzara differed from the

TABLE I

OUTBREAKS OF FILOVIRAL HEMORRHAGIC FEVER[a]

Location	Year	Virus/subtype[b]	Cases (mortality)	Epidemiology
Germany/Yugoslavia	1967	Marburg	32 (23%)[c]	Imported monkeys from Uganda source of most human infections
Zimbabwe	1975	Marburg	3 (33%)[d]	Unknown origin; index case infected in Zimbabwe; secondary cases were infected in South Africa
Southern Sudan	1976	Ebola/Sudan	284 (53%)[e]	Unknown origin; spread mainly by close contact; nosocomial transmission and infection of medical care personnel
Northern Zaire	1976	Ebola/Zaire	318 (88%)[f]	Unknown origin; spread by close contact and by use of contaminated needles and syringes in hospitals
Tandala, Zaire	1977	Ebola/Zaire	1 (100%)[g]	Unknown origin; single case in missionary hospital; other cases may have occurred nearby
Southern Sudan	1979	Ebola/Sudan	34 (65%)[h]	Unknown origin; recurrent outbreak at the same site as the 1976 outbreak
Kenya	1980	Marburg	2 (50%)[i]	Unknown origin; index case infected in western Kenya died; a physician secondarily infected survived
Kenya	1987	Marburg	1 (100%)[j]	Unknown origin; expatriate traveling in western Kenya

USA	1989	Ebola/Reston	4 (0%)[k]	Introduction of virus with imported monkeys from the Philippines; four humans asymptomatically infected
Italy	1992	Ebola/Reston	0 (0%)[l]	Introduction of virus with imported monkeys from the Philippines; no human infections associated
Ivory Coast	1994	Ebola/(Ivory Coast?)	1 (0%)[m]	Contact with chimpanzee; single case
Kikwit, Zaire	1995	Ebola/Zaire	315 (77%)[n]	Unknown origin; course of outbreak as in 1976
Gabon	1995/96	Ebola	37 (57%)[o]	Contact with chimpanzee

[a] Beside the well-documented episodes listed in this table two more suspected fatal and two nonfatal cases of Ebola hemorrhagic fever including a laboratory infection have been reported (165, 167, 168).

[b] Subtypes of Marburg are not classified.

[c] (127), numbers include a primary case which was diagnosed some years after the epidemic (W. Slenczka, unpublished data).

[d] (12).

[e] (22).

[f] (23)

[g] (166).

[h] (25).

[i] (13).

[j] E. D. Johnson, unpublished data.

[k] (29, 30).

[l] (40).

[m] (26).

[n] (27, 28).

[o] (169).

TABLE II

DISTRIBUTION OF CASES BY TRANSMISSION ROUTE DURING FOUR MAJOR EPIDEMICS

Transmission type	Germany/ Yugoslavia, 1967[a]	Sudan, 1976[b]	Zaire, 1976[c]	Sudan, 1979[d]
Nosocomial	4 (12.5%)	6 (2.1%)	85 (26.7%)	4 (11.8%)
Person-to-person	1 (3.1%)	231 (81.3%)	149 (46.9%)	27 (79.4%)
Nosocomial or person-to-person	1 (3.1%)	18 (6.3%)	43 (13.5%)	—[e]
Contact with infected monkeys	26 (81.3%)[f]	—	—	—
Neonatal	—	—	11 (3.5%)	—
Unknown	—	29 (10.2%)	30 (9.4%)	3 (8.8%)
Total number of cases	32 (100%)	284 (100%)	318 (100%)	34 (100%)
Total number of deaths (mortality)	7 (21.9%)	151 (53.2%)	280 (88.1%)	22 (64.7%)

[a] Based on (127).
[b] Based on (22).
[c] Based on (23).
[d] Based on (25).
[e] —, no data available.
[f] Including one primary case which was diagnosed some years after the epidemic (W. Slenczka, unpublished data).

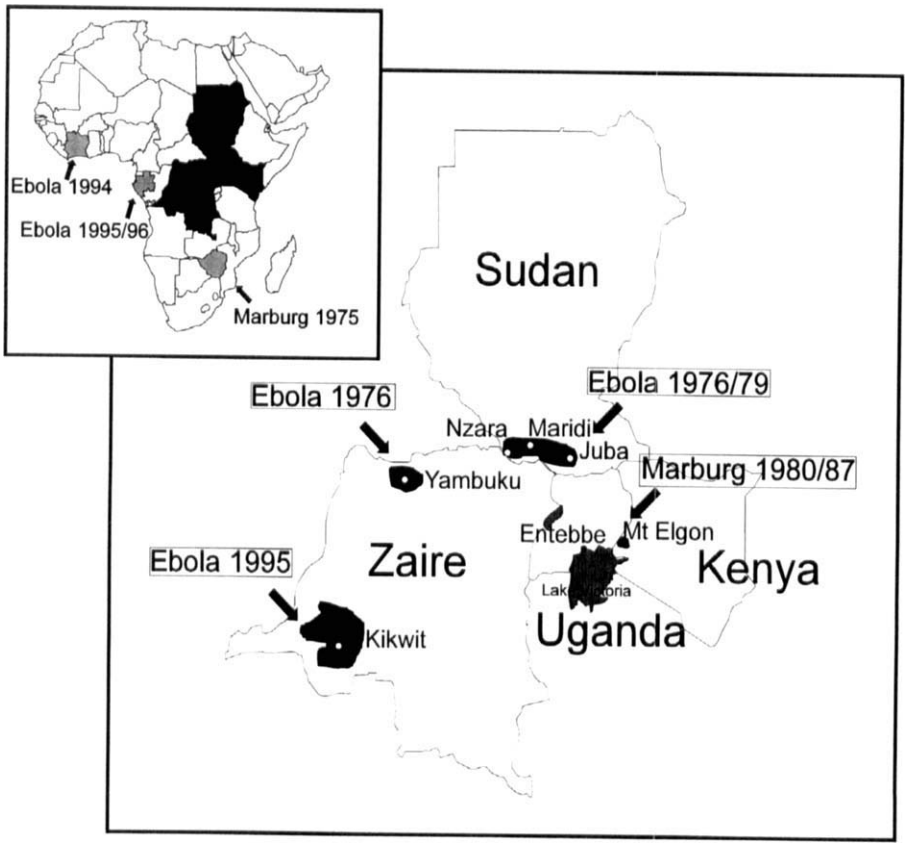


FIG. 1. Epicenters of hemorrhagic fever caused by filoviruses. Shown are the countries Kenya, Sudan, Uganda, and Zaire. The centers of the three major outbreaks of hemorrhagic fever caused by Ebola viruses are marked by black zones (Nzara, Maridi, and Juba, 1976 [22, 24, 25]; Yambuku, 1976 [23]; Kikwit, 1995 [27, 28]). Another black zone (Mount Elgon) indicates the region in which two index cases of Marburg hemorrhagic fever became infected (1980, 1987) (13; E. D. Johnson, unpublished data). A central holding station for wild-caught monkeys in the late 1960s was located near Entebbe at Lake Victoria. Infected vervet monkeys were shipped from here to Germany and Yugoslavia in 1967 (127). Three more episodes of filoviral hemorrhagic fever are indicated in the inset (gray): Marburg (Zimbabwe) 1975 (12), Ebola (Ivory Coast) 1994 (26), and Ebola (Gabon) 1995/96 (169).

one in Maridi. The Nzara outbreak involved factory workers and their close relatives whereas in Maridi the hospital served as both focus and amplifier of the infection. The outbreak lasted until November, during which time approximately 15 generations of person-to-person transmissions occurred (Table II). Transmission of the disease required close

contact with an acute case and was usually associated with nursing patients. The overall secondary attack rate was 12% and documented the relatively slow rate of spread into the community once out of the hospital. *In toto* there were 284 probable and confirmed cases involved with 151 deaths (53%) (Tables I and II).

By the end of August a second epidemic started in equatorial rain forest areas of northern Zaire (Fig. 1). A direct link between the two epidemics has always been discussed but never been verified. In total there were 318 probable or confirmed cases and 280 deaths (88%) (Tables I and II). The presumed index case came to Yambuku Mission Hospital for treatment of acute malaria, where he received an injection of chloroquine. It remains unclear whether this man was the source of the epidemic or became infected by the injection. Most persons acquired the disease following contact with patients, but for more than 25% the only risk factor elucidated was receipt of injections at Yambuku Mission Hospital (Table II). Nearly all survivors were infected by person-to-person contact. All ages and both sexes were affected, but the highest incidence was in women aged 15–29 years, who were frequently patients attending antenatal and outpatient clinics at the hospital. Although transmission was focused in the outpatient clinics of the hospital, there was subsequent dissemination in surrounding villages to people caring for sick relatives, attending childbirth, or having other forms of close contact. The secondary attack rate was approximately 5% overall but about 20% in close relatives of a patient. The epidemic, which lasted from the end of August until the end of October, spread relatively slowly in the epidemic area, and all infected villages (55; population <5000) were within 60 km of Yambuku.

Establishment of strict barrier nursing and classic public health principles, identification and isolation, was successful in controlling both epidemics. The spread by contaminated syringes and needles in Yambuku almost completely terminated when the hospital closed. The episode in Nzara died out spontaneously (14–23).

In 1979, Ebola hemorrhagic fever reemerged in Nzara and Yambio, which are located in the remote savanna of southern Sudan, near the border with Zaire (Table I; Fig. 1). The index case, a 45-year-old man, was admitted to the Nzara hospital with fever, vomiting, and diarrhea. He developed gastrointestinal bleeding and died 3 days postadmission. The index case worked in the same textile factory cited as the source of the 1976 outbreak in Sudan. The outbreak (July 31 to October 6, 1979) started from the hospital, where the index case patient was responsible for four nosocomial infections, which in turn led to disease in 5 families. Thirty-three cases could be traced to a human source of infection,

with 22 fatalities (65% mortality) (Tables I and II). Seven generations of virus transmission were estimated and mortality changed from 89% in the first four generations to 38% in the last three. Studies within the families confirmed reports from previous outbreaks (Sudan and Zaire, 1976) suggesting that Ebola virus is not easily transmitted and contact with body fluids of a patient is needed. Again, the hospital appeared to be the important focal point for dissemination (24, 25).

3. *New Emerging and Reemerging of Ebola Hemorrhagic Fever in Africa*

Two disease episodes of mortality were noticed among a group of chimpanzees in 1992 (8 deaths) and 1994 (12 deaths). The chimpanzees were objects of a 15-year observation by ethologists in the Tai National Park in western Ivory Coast. Several of the dead animals showed signs of hemorrhages, and one of the animals was autopsied in the field. A 34-year-old woman developed a dengue-like syndrome 8 days after performing the autopsy. She was admitted to the hospital in Abidjan 2 days later with continuing fever resistant to antimalarial treatment, diarrhea, and pruritic rush. Evacuation to Switzerland followed 5 days later when she developed a syndrome similar to that described for surviving Ebola-infected patients; she recovered without sequelae. An infection with an Ebola virus was confirmed by isolate-specific IgM and IgG antibodies, Ebola-Zaire-specific IgG antibodies, antigen-capture ELISA, reactivity to an Ebola serotype-specific monoclonal antibody, and virus isolation. Contact with infectious blood and tissues during the necropsy was considered to be the most likely source of the human infection. Organs of the dead chimpanzee were studied by immunohistochemistry and the findings were similar to those seen in material of the 1976 Ebola outbreaks and experimentally infected monkeys with Ebola virus. None of the persons in contact with either the case patient or the material of the chimpanzee tested antibody-positive (Table I; Fig. 1) (26).

Ebola hemorrhagic fever reemerged in Zaire in 1995. The first identified case related to the outbreak suffered an onset of illness on January 6, 1995 (Table I; Fig. 1). Until August 24, the official end of the epidemic, 315 cases had occurred, of which 244 died (77%). The center of the epidemic was Kikwit and the surrounding areas in Bandundu region in southwestern Zaire. The first case at Kikwit General Hospital was a male laboratory worker who had previously been admitted to Kikwit 2, a smaller second hospital in town. A laparotomy was performed after a differential diagnosis of typhoid fever with intestinal perforation. This was followed by a second laparotomy which showed

massive intraabdominal hemorrhage; the patient died 3 days later. Four days after the first laparotomy the first case among medical staff members occurred, with fever, headache, muscle aches, and hemorrhages. About three quarters of the first 70 patients were health care workers. The actual epidemic started within the hospitals. Prior to this time, cases had been sporadic. The major risk factors have been patient care in hospitals and households and preparation of bodies for burial. This is reflected by the fact that 26% of the cases with known professional occupation were medical staff members or students and 21% were housewives. During the course of surveillance, several chains of deaths have been identified which were traced as far back as late December 1994. The chain of the presumable index case, a charcoal worker, involves 7 out of 12 persons living in his household (27, 28).

An outbreak of Ebola hemorrhagic fever occurred in the village of Mayibout II, Makokou Health District, Ogooue-Ivindo Province, Gabon. It was linked to a chimpanzee found dead in the forest. A total number of 37 cases were diagnosed (mortality 56.8%) and 21 cases were directly exposed to the dead chimpanzee. A strain of Ebola virus was isolated from patient samples (169).

4. Reston Hemorrhagic Fever

In 1989, veterinary staff in a primate import quarantine facility in Reston, Virginia noted numerous deaths in cynomolgus monkeys in one animal room and suspected simian hemorrhagic fever (SHF) (Table I). Samples tested in the virus laboratory yielded SHF, but a filovirus closely related to Ebola was also isolated from those monkeys; the agent was called Ebola Reston (29–31). These cynomolgus monkeys (*Macaca fascicularis*) were imported from the Philippines. Shipments arrived either via Amsterdam or directly from the Philippines via the Pacific Ocean. No link to African or animals of other continents could be established on any route, so the presumption prevails that this new Ebola virus isolate is of Asian origin. The role of SHF in initiating or propagating the epidemic is unknown, but the filovirus was found to be pathogenic for monkeys under experimental conditions. Filoviral antigen and particles were found in tissues of naturally and experimentally infected monkeys in close anatomic relationship to the pathologic lesions (30–33). The epizootic occurring in monkeys spread through affected rooms by droplet contact with adjacent cages or to distant cages and different rooms by larger droplets and/or small-particle aerosols (34, 35). An airborne route of transmission was supported by the prominent respiratory involvement of the infected monkeys. Spread of the disease to other rooms of the facility lead to a decision to euthanize

all the monkeys in the building. Resumption of importation of monkeys led to new outbreaks of disease; subsequent investigation traced the source to a single source in the Philippines that was thought to have furnished all identified infected shipments, including monkeys sent to facilities in Texas and Pennsylvania (36). Four animal handlers at the quarantine facility became infected as judged by serological tests and, in one case, virus isolation. All four had high levels of daily exposure, but except for one, who cut himself while performing a necropsy, the mode of transmission is unclear (37–39). None of them had an unexplained febrile illness, suggesting that this virus may be less pathogenic for humans than previously known filoviruses, which have resulted in significant disease and mortality rates ranging from 22 to 88%. However, these observations should not be interpreted as assuring that this virus is not virulent for humans.

There is evidence that the outbreak in 1989 was not confined to the Reston facility, but occurred also in a branch in Münster, Germany, of the same company. However, since material has not been made available for laboratory diagnosis the nature of this agent remains uncertain.

In 1992, cynomolgus monkeys were imported into Italy from the same holding compound in the Philippines that exported the monkeys causing the 1989–1990 epizootic (Table I). Ebola Reston virus was isolated from three monkeys which died, and the remaining animals were sacrificed thereafter. No illness in associated humans has been reported (40). Reportedly, in 1991 monkeys were eliminated from the holding facility and cages disinfected. Thus, the virus either had persisted or was reintroduced by a similar mechanism as led to the 1989–1990 epizootic. Since the facilities in the Philippines were extensively cleaned following the first episode and since the isolated virus belonged to the EBO Reston subtype, it appears that reintroduction occurred from wild-caught animals of the same or a distinct endemic area.

Evidence for ongoing epizootic disease and transmission among captured monkeys at the export facility in the Philippines, which was the source of several shipments to the United States (1989–1990) and the shipment to Italy (1992) that contained infected monkeys, was demonstrated in 1990 and 1993. In 1990, filoviral antigen was detected by ELISA in 52.8% of dead monkeys from this facility in contrast to none in the dead monkeys from another facility in the Philippines. The investigation suggested that the type of holding cage was important in transmission, since being in a gang cage at the time of the initial serosurvey was a significant risk factor for subsequent infection (36). In the summer of 1993, high titered ELISA antibodies were present in monkeys held at that facility, but no evidence of viral antigen was found. Monkeys

imported from the facility into the United States in 1993 had stable IgG titers, suggesting infection in the recent past but not during quarantine (4). Even so, the original source remains unknown. It seems likely that naturally infected wild monkeys captured in the Philippines are the source of introduction.

B. Reservoir

Serological studies suggest that filoviruses are endemic in many countries of the Central African region (Fig. 2) (41–53). Recent serosurveys in other countries, such as Germany and the United States (54, 55), using several different techniques, suggest that filoviruses might also be endemic in those countries. Serological studies in relation to the EBO Reston outbreak indicated filovirus activity in the Philippines (Fig. 2). Although, as already mentioned, serological data based on IFA alone are of limited reliability, they at least suggest that subclinical infections caused by known or unknown filoviruses may occur and may be more common than expected. At this point, however, one has also to consider that filoviruses are members of the order *Mononegavirales*. This order includes many common human viruses that could be responsible for serological cross-reactivities.

MBG and subtypes Sudan and Zaire of EBO appear to be indigenous to the African continent, and both EBO subtypes have been isolated from human patients only in Africa. MBG has been isolated from human patients in Africa and Europe. The origin of the European cases could be traced back to foci in Uganda where vervet monkeys compounded in Entebbe (central holding station at Lake Victoria) were imported to Germany and Yugoslavia (Fig. 1). Complement-fixing antibodies were found in sera from some monkeys originally trapped near Lake Kyoga, the main area where vervet monkeys had been captured since the establishment of the trade in 1962. The finding of antibodies in three monkey trappers indicates that human infection may have occurred in Uganda during that time. However, all titers observed were weak, and an agent has never been isolated from the blood of a wild-trapped monkey or a monkey trapper (10, 56). Both index case patients of the episodes of MBG hemorrhagic fever in Kenya (1980/1987) had traveled in Mount Elgon region (Fig. 1). This region is not far from the shores of

FIG. 2. Prevalence of filovirus-reactive antibodies. *Key:* black, countries that have been subject to published serosurveys based mainly on IFA; gray, countries that have been subject to serosurveys based on different techniques. For references see Section II,B and reference list.

Lake Victoria and thus is close to the trapping place (Lake Kyoga, Uganda) and holding station (Entebbe, Uganda) of the monkeys that initiated the 1967 outbreak in Europe. One of the index cases had visited a cave (Kitum Cave) in that area shortly before becoming ill. Serological studies in this area, however, again failed to uncover the source of the virus. These studies included an extensive investigation of many animal species inhabiting the cave, including bats (E. D. Johnson, unpublished). Bats inhabiting buildings of a textile factory have also been considered as a potential source of the index cases of the 1976 and 1979 outbreaks of EBO hemorrhagic fever in southern Sudan. The geographical origin of both epidemics is less than 1000 km northwest of Lake Victoria and Mount Elgon (Fig. 1). A potential source for the 1976 Zairian outbreak has never been found, but a link to the simultaneous epidemic in Sudan has been discussed. These data strongly suggest an endemic focus for filoviruses in the equatorial rain forest areas of southern Sudan, northern Zaire, Uganda, and Kenya (Fig. 1).

The EBO Reston outbreak suggested for the first time the presence of a filovirus in Asia. Serological studies (IFA) among captive macaques in the Philippines indicated that the source of EBO Reston might be wild nonhuman primates. However, IFA-detected antibodies seem to be spurious, and latent infection in nonhuman primates has never been observed (57). Epidemiological data obtained in association with the 1994 Ivory Coast case suggested an Ebola epizootic among a group of chimpanzees as the cause of death. The pathogenicity of filoviruses, especially of EBO subtypes Sudan and Zaire and MBG, for nonhuman primates, however, does not support the concept of a reservoir in monkeys.

The reservoir of filoviruses remains a mystery. Many species have been discussed as possible natural hosts; however, no nonhuman vertebrate hosts or arthropod vectors have yet been identified. The high frequency of false-positives, especially when the EBO IFA is used, has contributed to the difficulties in finding the true reservoir for filoviruses. Filoviruses resemble "Old World" arenaviruses in several interesting biological properties, such as resistance to the antiviral effects of interferon, lack of *in vivo* neutralization, and lack of protection by convalescence sera. Arenaviruses cause chronic viremic infection in their rodent reservoirs. Thus, chronic infection of a mammal has to be considered as a mechanism that regulates survival of filoviruses in nature.

C. Transmission

Person-to-person transmission by physical contact with case patients is the main route of infection in human outbreaks (Table II). Activities such as nursing and preparing bodies for burial are espe-

cially associated with an increased risk of becoming infected. During the EBO outbreaks in 1976 and to some extent 1995, nosocomial transmission via contaminated syringes and needles was a major problem. Transmission does not seem to be efficient, as documented by secondary attack rates which on average rarely exceeded 12%. Thus, extreme care should be taken with blood, secretions, and excretions of infected patients. Sexual transmission has been described for MBG (58) and neonatal transmission has been reported for the 1976 outbreak in Zaire (16). Based on experience of the former episodes, isolation of patients and use of strict barrier nursing procedures (*e.g.*, protective clothing, respirator) are sufficient to interrupt transmission. Transmission by droplets and small-particle aerosols has been observed among experimentally infected (MBG) and quarantined imported monkeys (EBO RES, 1989–1990) (34, 59, 60). This is confirmed by identification of filovirus particles in alveoli of naturally and experimentally infected monkeys (33, 34, 61, 62) and human post mortem cases (63). However, the contribution of aerosol transmission to the course of human outbreaks is still unknown.

D. Molecular Evolution

The family *Filoviridae* has been constituted on the basis of unique morphologic, morphogenetic, physicochemical, and biological features of its members (64). Filoviruses can be separated into two types, which are clearly distinguished by the features listed in Table III. In general, the MEG viruses seem to be unique without known subtypes, but at least two different genetic lineages coexist (65). EBO, however, can be subdivided into at least three subtypes: Zaire, Sudan, and Reston (66–69). Molecular characterization of the Ivory Coast virus revealed a novel lineage, suggesting a fourth subtype of EBO (Fig. 1) (69). There is a lack of antigenic cross-reactivity between the types, but the subtypes of EBO share common epitopes (68, 70). Nucleotide sequence comparison among MBG and EBO shows only scattered similarities, which is in contrast to the similarities seen among amino acid sequences of structural proteins (71–73). This finding indicates that these agents may have diverged at some point in the distant past. A distinction within the EBO type is based on earlier peptide and oligonucleotide mapping (74, 75) and has been confirmed by recent sequence analysis of the glycoprotein genes. That study showed all four subtypes to differ from one another to a comparable extent: 37–41% nucleotide differences (69). This suggests that filoviruses have evolved into specific niches and may reflect a similar divergence in the natural hosts, assuming they have coevolved. Genetic variability seen among different virus isolates of one subtype seems to be much less than for some other RNA viruses.

TABLE III

CHARACTERISTICS OF MARBURG AND EBOLA TYPES OF FILOVIRUSES

Features	Type Marburg	Type Ebola
Serological cross-reactivity to other type	No	No
Subtypes	1[a]	3[b]
Glycoprotein (SDS-PAGE)[c]	About 170 kDa	About 140 kDa
Terminal sialylation of carbohydrates[d]	No	Yes
Nucleoprotein (SDS-PAGE)	About 95 kDa	About 105 kDa
Nonstructural proteins	No	1[e]
Editing	No	Yes[f]
Gene overlaps	1[g]	>1[h]
Overlapping ORF[i] in gene 2	Yes	No

[a] Type Marburg: subtype Marburg.
[b] Type Ebola: subtypes Zaire, Sudan, Reston.
[c] SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
[d] By propagation of viruses in Vero E6 and MA-104 cells (monkey kidney cell lines).
[e] Small glycosylated protein encoded by gene 4.
[f] Gene 4.
[g] Between VP30 and VP24.
[h] Between VP35 and VP40, GP and VP30, and VP24 and L (Ebola, subtype Zaire).
[i] ORF, opening reading frame.

In particular, the close genetic relation of the two Zairian isolates from 1976 and 1995, with less than 1.6% difference in the GP gene (69), suggests that filovirus variants may not emerge as rapidly in nature.

Molecular analyses of the genomes clearly demonstrated that filoviruses are the closest relatives to *Rhabdoviridae* and *Paramyxoviridae* (Fig. 3). All nonsegmented negative-stranded (NNS) RNA viruses share a similar genome organization, with conserved regions at both ends encoding the core and L proteins surrounding a variable part in the middle encoding the envelope proteins (Fig. 3B). Filovirus genomes are more complex than those of lyssaviruses and vesiculoviruses and align organizationally more closely to members of the genera *Paramyxovirus* and *Morbillivirus*. This relationship is confirmed on the amino acid level, as demonstrated for the nucleoproteins and polymerases (L proteins) (67, 71). Transcription and replication of all NNS RNA viruses, including filoviruses, follow common principles, such as (i) helical ribonucleocapsid complex as the functional template for synthesis of replicative and messenger RNA, (ii) transcription of messenger RNAs by sequential interrupted synthesis from a single promoter at the 3′ genomic end, (iii) replication via a full-length anti-

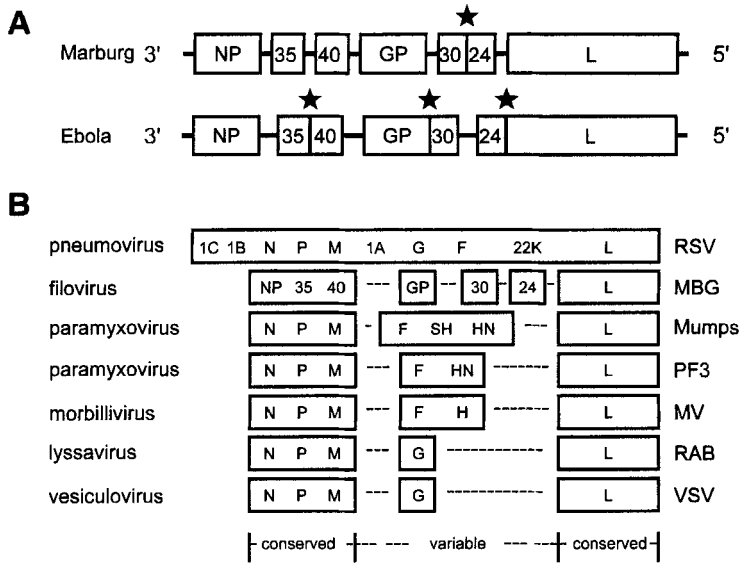


FIG. 3. (A) Genome organization of filoviruses. Filoviral genomes consist of a single, negative-stranded, linear RNA molecule. Differences in organization between Marburg and Ebola type viruses are indicated. Key: asterisk, position of gene overlap; GP, glycoprotein gene; L, polymerase (L) gene; NP, nucleoprotein gene; 24 / 30 / 35 / 40, virion structural protein (VP) genes. (B) Genome organizations of nonsegmented negative-stranded RNA viruses. Compared are genomes of viruses belonging to different genera of the three families Paramyxoviridae, Rhabdoviridae, and Filoviridae (Order *Mononegavirales*). Conserved and variable regions are identified. *Key:* RSV, human respiratory syncytial virus; MBG, Marburg virus; Mumps, mumps virus; PF3, human parainfluenza 3 virus; MV, measles virus; RAB, rabies virus; VSV, vesicular stomatitis virus; N and NP, nucleoprotein gene; P, phosphoprotein gene; 35, virion structural protein (VP) 35 gene, the putative P equivalent; M, matrix protein gene; 40, VP40 gene, the putative M equivalent; G, GP, F, H, HN, glycosylated membrane protein genes; SH, small hydrophobic protein gene; 22K, nonglycosylated membrane protein gene; 30, VP30 gene, unknown function; 24, VP24 gene, unknown function; L, polymerase gene.

genome of positive sense, (iv) transcription and replication in the cytoplasm, and (v) maturation by envelopment of independently assented ribonucleocapsid complexes at membrane sites containing viral proteins. This is reflected by common genomic features such as complementarity of the genome termini homologies in the 3′ leader regions, conservation of transcriptional signals, separation by intergenic sequences, and expression of virion-associated RNA-dependent RNA polymerases. In conclusion, all data available today support the concept of an order *Mononegavirales* comprising the three unique families *Paramyxoviridae, Rhabdoviridae,* and *Filoviridae* (76).

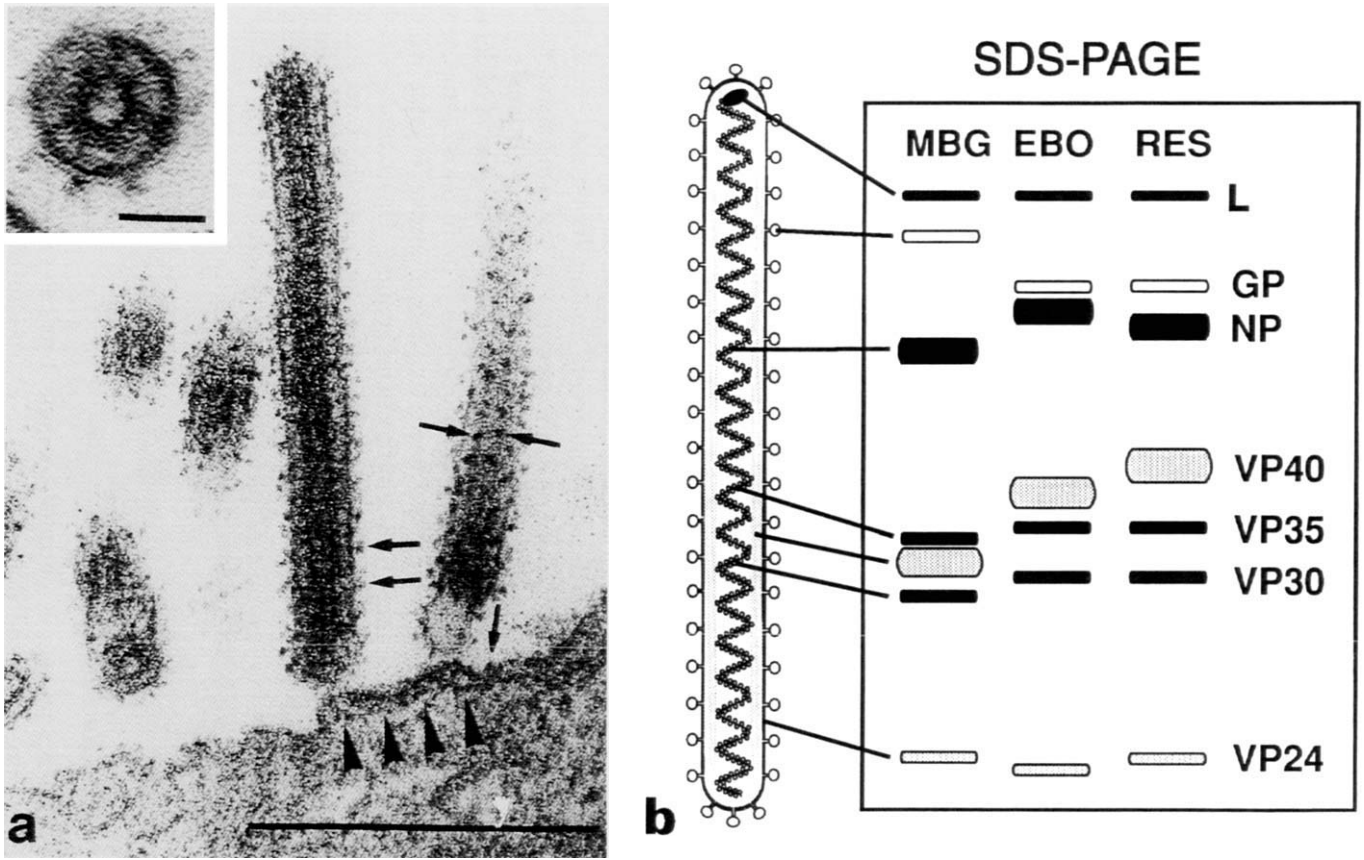
III. Biology of Filoviruses

A. Morphology

The long filamentous shape of the particles is unique among viruses and has been decisive for classification. Particles appear in different shapes, such as branched, circular, or U- and 6-shaped forms. Virions vary greatly in length but show a uniform diameter of approximately 80 nm. Family members differ in length of virion particles but seem to be very similar in morphology. Peak infectivity has been associated with particles of 665 nm for MBG and 805 nm for EBO. Virions are composed of a central core formed by a ribonucleocapsid complex (RNP) which is surrounded by a lipid envelope derived from the host cell plasma membrane. Electron micrographs demonstrate an axial channel (10–15 nm in diameter) within the RNP. The channel is surrounded by a central dark layer (20 nm in diameter) and an outer helical layer (50 nm in diameter) with cross-striations at 5 nm intervals. Spikes approximately 7 nm in length and spaced at about 10 nm intervals form globular structures on the virion surface (Fig. 4A) (19, 58, 64, 77–79).

The RNP is composed of a single molecule of linear RNA and four of the seven virion structural proteins [nucleoprotein (NP), VP30, VP35, and the large (L) protein]. Genomic RNA has an M_r of 4.2×10^6 and constitutes 1.1% of the virion mass (80). The three remaining structural proteins are membrane-associated, with the glycoprotein (GP) as a type I transmembrane protein (81) and VP24 and VP40 probably located at the inner side of the membrane. Virus particles have an M_r of approximately $3–6 \times 10^8$ and a density in potassium tartrate of 1.14 g/cm^3 (Fig. 4B) (66).

Fig. 4. Morphology of filoviral particles. (A) Electron micrograph. Budding of Marburg virus particles from the plasma membrane of infected primary cultures of human endothelial cells. Particles consist of a nucleocapsid surrounded by a membrane in which spikes are inserted (*arrows*). The nucleocapsid contains a central channel (*inset*). The plasma membrane of infected cells is often thickened at locations where budding occurs (*arrowheads*). Ultrathin section—bar, 0.5 μm; bar inset, 50 nm (116). (B) Electrophoretic mobility patterns of filoviral structural proteins. The mobility patterns (SDS-PAGE) of structural proteins of Marburg (MBG, strain Musoke) and two Ebola subtypes, Zaire (EBO) and Reston (RES), are compared and differences illustrated. Four proteins are involved in nucleocapsid formation: polymerase or large (L) protein, nucleoprotein (NP), virion structural protein (VP) 30 and VP35 (*black*). The glycoprotein (GP) is a transmembrane protein and anchored with the carboxy-terminal part in the virion membrane (*white*). Homotrimers of GP form the spikes on the virion surface [*arrows* in (A)]. VP40 and VP24 are membrane-associated proteins (*gray*).


a
b
SDS-PAGE
MBG
EBO
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B. Genome

Genomes of filoviruses consist of a single negative-stranded linear RNA molecule (64, 80, 82). The RNA is noninfectious, not polyadenylated, and complementary to polyadenylated viral subgenomic RNA species (64, 72, 83). The nucleic acid sequences of two different isolates of MBG (83, 84) and the EBO Mayinga isolate (subtype Zaire) (72, 85) as well as parts of EBO Reston and EBO Maridi and Nzara isolates (subtype Sudan) (69; A. Sanchez, personal communication) have been elucidated. Filovirus genomes have a length of approximately 19 kb (19.1 for MBG and 18.9 kb for EBO) and are larger than all other negative-stranded RNA virus genomes. Genes have been identified by highly conserved transcriptional signals at their 3′ and 5′ ends. The following order is characteristic for filoviruses: 3′ leader–NP–VP35–VP40–GP–VP30–VP24–L–5′ trailer (Fig. 3A).

Genes are separated by intergenic regions varying in length and nucleotide composition. Some genes overlap but the positions and numbers of overlaps are different among filoviruses (Fig. 3A; Table III). Viruses belonging to the Zairian subtype of EBO possess three overlaps located between VP35 and VP40, GP and VP30, and VP24 and L, whereas MBG isolates have only one overlap between VP30 and VP24. The length of the overlaps is limited to five highly conserved nucleotides within the transcriptional signals (3′–UAAUU–5′) (Fig 5). Transcriptional start signals are conserved among filoviruses, and the sequence 3′–CUNCNUNUAAUU–5′ represents the consensus motif (Fig. 5). Transcriptional stop signals are identical for all genes (3′–UAAUUCUUUUU–5′) with the exception of the VP40 gene of MBG (C at position 2 instead of an A; genomic sense) (Fig. 5). Most genes tend to possess long noncoding sequences at their 3′ and/or 5′ ends which contribute to the increased length of the genome. Upstream of the N gene start site and downstream of the L gene stop site there are extragenic sequences, which are thought to be templates for small viral, nonpolyadenylated subgenomic RNAs synthesized during infection. The genomes are complementary at the very extreme ends, a feature known for all NNS RNA viruses (83, 85, 86).

C. Viral Proteins

1. NP—Nucleoprotein

The NP protein is encoded by gene 1 at the extreme 3′ end of the linear unsegmented RNA genome. NP proteins differ slightly in their electrophoretic mobility patterns, ranging from 95 kDa for MBG to 105

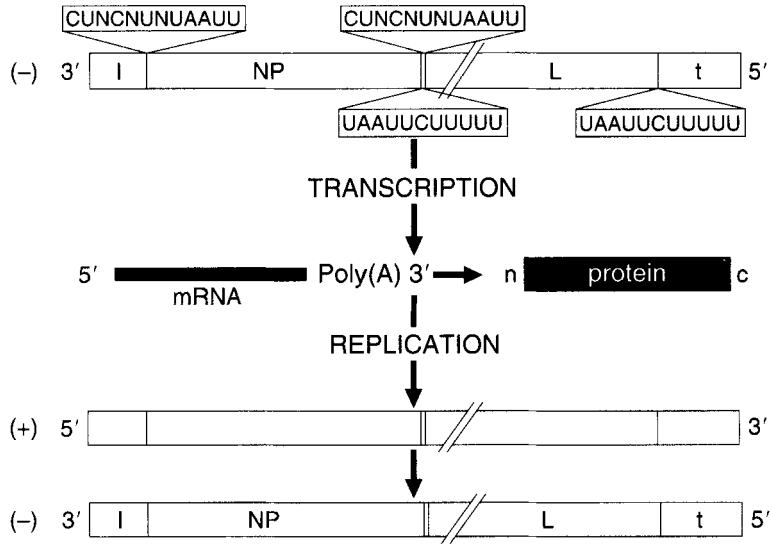

FIG. 5. Replication cycle of filoviruses. The model illustrates the mode of transcription and replication based on the data available to date. Each gene on the linear arranged nonsegmented (–)-sense genome is flanked by conserved transcriptional start (3′–CUNCNUN*UAAUU*–5′; indicated above) and termination signals (3′–UAAUUCUUUUU–5′; indicated beneath). Transcription starts at the 3′ end of the (–)-sense genome and leads to polyadenylated mRNA species. For replication a full-length (+)-sense antigenome is synthesized which serves as the template for the synthesis of progeny (–)-sense RNA anticomplementary to the parental RNA. *Key:* c, carboxy-terminal end of proteins; l, 3′ untranslated region (leader); n, amino-terminal end of proteins; Poly(A), polyadenylation of mRNA species; t, 5′ untranslated region (trailer); NP, nucleoprotein; L, viral RNA-dependent RNA polymerase.

kDa for EBO isolates. The molecular weights (MW) calculated from the deduced amino acid sequences of the corresponding genes of MBG (695 amino acids) and EBO (739 amino acids) are 78 kDa and 83 kDa, respectively, and the differences in lengths are related to the less-conserved C termini of the protein (71, 83, 84, 87). Thus, filovirus NP proteins possess an unusually high MW compared with other NNS-RNA virus nucleocapsid proteins, which range from 42 to 62 kDa. This suggests additional functions for the filovirus NP protein located in its unique C terminus. The NP protein is the major structural phosphoprotein, and only the phosphorylated form of the protein is incorporated into virions, as demonstrated for MBG (88–90). This finding may indicate that phosphorylation is needed to interact with genomic RNA and to form virion RNPs for progeny viruses. Sequence comparison of

NP proteins of MBG and EBO shows a high degree of homology within the first 400 predicted amino acids. The alignment shows that the region from position 130 to 392 of the MBG sequence has a very strong similarity and is highlighted by a run of 34 identical amino acids from position 296 to 329. The fact that two of the three cysteine residues of NP are conserved may indicate their role in proper folding of the molecule. A small region in the middle of the MBG and EBO NP sequences was found to contain a significant amino acid homology with paramyxoviruses and to a lesser extent with rhabdoviruses (71). The NP proteins of filoviruses and other NNS viruses also have the hydrophobicity of their N termini in common. A role of this region in either protein folding and/or RNA binding has been postulated for other NNS-RNA viruses (91, 92). The less-conserved C-terminal half of filovirus NP proteins, which is hydrophilic and very acidic, may function in the assembly process by interacting with the matrix proteins or the presumed second proposed nucleoprotein VP30 (66, 71, 88). Similar functions have been discussed before for the variable C termini of paramyxovirus NPs (91, 93). The NP protein is the major component of the RNP and is tightly bound within the complex. Although RNA binding has not yet been demonstrated, there is little doubt that this protein is the functional analogue of the nucleocapsid proteins of paramyxoviruses and rhabdoviruses (Fig. 4B; Table IV).

2. VP35—Polymerase Cofactor?

VP35 is encoded by gene 2. It has a length of 329 amino acids in MBG (73, 83); VP35 of EBO is 351 or 340 amino acids long (72, 73, respectively). RNP association of the protein is much weaker than that of NP and VP30, as demonstrated by nonionic detergent treatment of virion particles (66, 88). VP35 of EBO virions is not phosphorylated (88, 89). Expression studies of MBG VP35 in insect cells (SF9 cells), however, revealed weak phosphorylation of this protein, whereas VP35 expressed in HeLa cells using the vaccinia-virus-driven T7 polymerase system was not phosphorylated (H. Feldmann, unpublished data). Thus, VP35 may exist in a phosphorylated and an unphosphorylated form as has been demonstrated for NP. For NP, the unphosphorylated form seems to be incorporated into virion particles. Hydropathy plots of MBG and EBO VP35 showed similar profiles and a prominent common hydrophilic domain in close proximity to the N termini (MBG, positions 28 to 42; EBO, positions 57 to 76). This region may be involved in template binding; this is supported by the fact that VP35 unspecifically binds nucleic acids (H. Feldmann, unpublished data). In spite of the

TABLE IV

FILOVIRAL PROTEINS AND THEIR PROPOSED FUNCTION[a]

Designation	Virus type	Encoding gene	Localization	Proposed function
NP	MBG/EBO	1	Ribonucleocapsid complex	Encapsidation
VP35	MGB/EBO	2	Ribonucleocapsid complex	Phosphoprotein analogue
VP40	MBG/EBO	3	Membrane-association	Matrix protein
GP	MBG/EBO	4	Surface (transmembrane protein)	Receptor binding, fusion
VP30	MBG/EBO	5	Ribonucleocapsid complex	Encapsidation, RNA binding
VP24	MBG/EBO	6	Membrane-association	Unknown
L	MBG/EBO	7	Ribonucleocapsid complex	RNA-dependent RNA polymerase
sGP	EBO	4[b]	Nonstructural, secreted	Unknown

[a] NP, nucleoprotein; VP, virion structural protein; GP, glycoprotein; L, large protein (polymerase); sGP, small glycoprotein; MBG, type Marburg filoviruses; EBO, type Ebola filoviruses.

[b] Expressed by RNA editing (69, 112).

inconsistent data on phosphorylation among filoviruses and the lack of sequence homology, the genome position of the corresponding gene combined with the association in the RNP suggest that VP35 is functionally analogous to the P proteins of paramyxoviruses and rhabdoviruses. Further studies are needed to show if "P" protein would be an appropriate designation for this protein (Fig. 4B; Table IV).

3. VP40—Matrix Protein

VP40 of filoviruses is encoded by gene 3. It is 303 and 326 amino acids long in MBG and EBO, respectively (72, 73, 83). Differences in the electrophoretic mobilities of VP40 can be used to distinguish between MBG and EBO isolates and even among the three EBO subtypes (Fig. 4B) (68). Nitrocellulose-bound VP40 binds in a radiooverlay protein assay unspecifically to nucleic acids (H. Feldmann, unpublished data),

implicating a role in regulation of transcription/replication as has been described for the matrix protein of vesicular stomatitis virus (94, 95). VP40 is not associated with the RNP complex and behaves like a membrane-associated protein when analyzed following nonionic detergent treatment of virion particles (66, 88). This finding, together with a predominantly hydrophobic profile, the abundance in virion particles, and the genome localization of the corresponding gene, suggests that VP40 is the matrix protein analogue of filoviruses (Fig. 4B; Table IV).

4. GP—Glycoprotein

GP, encoded by gene 4 of the genome, is the only glycosylated structural protein of virions. GP of MBG and EBO is 681 and 676 amino acids in length, respectively (72, 81, 96, 97). Filovirus GPs are type I transmembrane proteins anchored via a C-terminal hydrophobic domain in the membrane (Fig. 6A). They are directed into the endoplasmic reticulum by an N-terminal hydrophobic domain which shows the structural requirements for signal peptides and can be cleaved by signal peptidases as shown directly for the MBG GP (81). Filovirus GPs contain *N*- and *O*-glycans that account for up to 50% of the MW of the mature proteins. Oligosaccharide side chains differ in their terminal sialylation patterns, which seem to be isolate- as well as cell-line-dependent (68, 98). Detailed structural analyses of filovirus carbohydrates are available for MBG only. The structures include oligomannosidic and hybrid-type *N*-glycans as well as bi-, tri-, and tetraantennary complex species and high amounts of neutral mucin-type *O*-glycans (99). Amino acid sequence comparison of filovirus GPs showed conservation at the N- and C-terminal ends of the proteins in which the two hydrophobic domains (signal peptide, membrane anchor) and most of the highly conserved cysteine residues are located. The middle region is variable and extremely hydrophilic and carries the bulk of the glycosylation sites for *N*- (EBO 17 sites; MBG 22 sites) and *O*-glycans (Fig. 6A). Recently it has been shown that the two cysteine residues at positions 671 and 673 of MBG GP are acylated (100). Acylation at the border between membrane anchor region and cytoplasmic tail has been shown for many viral type I transmembrane proteins. The special arrangement of all cysteine residues in the molecule favors an intermolecular cysteine bridge formation between the two external parts of the molecule, resulting in a stem region with a crown-like domain on the top carrying the mass of the carbohydrate side chains (Fig. 6B). For MBG it has been shown that the mature GP is inserted in the membrane as a homotrimer, and oligomerization seems to be mediated by intramolecular disulfide bridges, since complexes can be destroyed

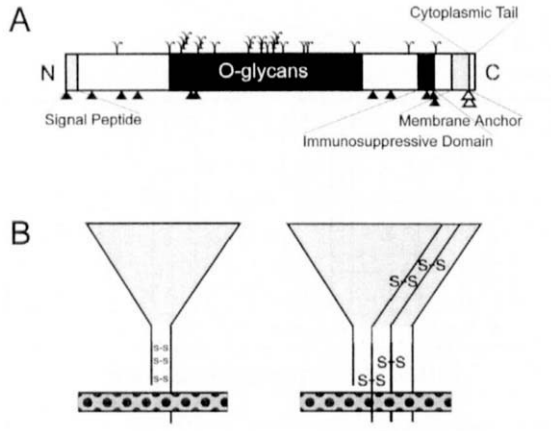

FIG. 6. (A) Schematic illustration of structural features of filoviral glycoproteins. The glycoprotein (GP) of filoviruses is encoded by gene 4. It is a type I transmembrane protein carrying two hydrophobic domains (*light gray*) at the amino-terminal (signal peptide) and carboxy-terminal ends (membrane anchor). An external domain in close proximity to the transmembrane region (*black*) shows significant homology to an immunosuppressive domain in envelope proteins of several retroviruses. The protein is highly glycosylated, carrying N- and O-linked oligosaccharides (γ) (here demonstrated for MBG strain Musoke). (B) Structural model of the surface spikes of filoviruses. The surface spikes of filoviral particles are formed by homotrimers of the glycoprotein (GP). Each monomer seems to form a stemlike structure with a crownlike domain on the top carrying the mass of the carbohydrate site chains. This formation may be mediated by intramolecular disulfide bridge formation (S–S) of cysteine residues located at the amino and carboxy termini of the protein [▲ in (A)]. Oligomerization seems to be due to intermolecular disulfide bridge formation (S–S) (98). *Key:* ▲, cysteine residues; △, acylated cysteine residues; γ, N-linked oligosaccharides.

under reducing conditions (Fig. 6B) (98). In general, filovirus GPs lack significant homologies with envelope proteins of other NNS RNA viruses. However, a region of 26 amino acids in the external domain in close proximity to the transmembrane region shows significant homology to an immunosuppressive domain in the envelope proteins of several retroviruses (Figs. 6A and 7) (72, 81, 96, 97). The immunosuppressive domain in retroviruses has been assumed to be involved in inhibition of blastogenesis of lymphocytes, decrease in monocyte chemotaxis and macrophage infiltration, inhibition of human natural killer-cell

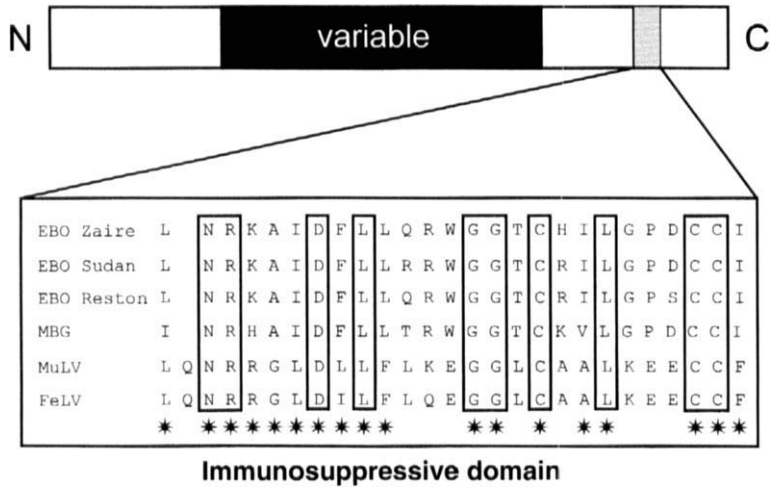

FIG. 7. Immunosuppressive motif located on filoviral glycoproteins. Shown is an alignment of the putative immunosuppressive motif (peptide of 26 amino acids) of the Zaire, Sudan, and Reston subtypes of Ebola viruses and Marburg virus (strain Musoke), compared with the known motif of two oncogenic retroviruses, murine leukemia virus (MuLV) and feline leukemia virus (FeLV). Identical amino acids are framed and functionally similar ones are identified beneath the alignment by asterisks. The motifs are located in close proximity to the transmembrane region on the conserved carboxy-terminal part of the molecules.

activity, and blocking of protein kinase C activity (101–103). Experimental data on GP function do not exist. However, the fact that GP is the only surface protein of the virions suggests a function in mediation of binding to cellular receptors and fusion with cellular membranes. Furthermore, GP is discussed as the major viral antigen and the main target for the host immune response (Figs. 4, 6, and 7; Table IV).

5. VP30—Minor Nucleoprotein?

VP30 is encoded by gene 5 and intimately associated with the RNP (66, 88). The protein has a length of 260 and 281 amino acids in EBO and MBG, respectively (72, 83, 84). The protein binds RNA under denaturing conditions in an RNA–protein overlay assay and forms complexes with the NP protein. Complexes of expressed recombinant VP30 and NP can be precipitated using either anti-VP30 or anti-NP antibodies (H. Feldmann, unpublished data). VP30 of EBO has been identified as the minor phosphoprotein of virions (88, 89). The available data indicate that VP30 may work as a functional unit in encapsidation of the

RNA genome (66), which could be achieved by binding to NP and/or binding to genomic RNA. It could also play a role as an additional cofactor of the transcriptase–replicase complex (Fig. 4; Table IV).

6. VP24—Membrane-Associated Protein of Unknown Function

VP24 is encoded by gene 6 of filoviruses. It is 253 and 251 amino acids in length in MBG and ERO, respectively (72, 83, 84). The protein is membrane-associated. Unlike VP40, it is not completely removed from the RNP under isotonic conditions (66, 88). VP24 presumably serves as a second matrix protein and may bind to the cytoplasmic tail of GP or may link the membrane proteins (VP40 and/or GP) to the RNP. There are minor differences in the SDS-PAGE migration profile of this protein among filovirus isolates (Fig. 4B). Such differences have also been observed between EBO wild type (subtype Zaire) and a highly pathogenic variant isolated after several passages from guinea pigs (104). To what extent these changes contribute to the higher pathogenic potential of the variant in guinea pigs is currently unknown (Fig. 4B, Table IV).

7. L—Large Protein

The L protein is encoded at the 5′ end of the linear genome and has a predicted MW of 267 kDa (2331 amino acids) for strains Musoke (105) and Popp (84) of MBG. Computer-assisted comparison revealed significant homologies to L proteins of other NNS RNA viruses. Homologies are mainly located in the N-terminal half of the protein and are concentrated within three common domains, named boxes A, B, and C (106). Other common features are a high content of leucine and isoleucine residues, a large positive net charge, clusters of basic amino acids, putative ATP binding sites, two neighboring cysteine residues located in the C-terminal half of the protein, and the genome localization of the encoding gene. A highly conserved peptide motif –GDNQ– located at the C-terminal end of domain B (positions 744–747) and flanked by hydrophobic amino acid residues seems to be correlated with enzymatic functions of the protein. Mutations in this domain, which is present in all NNS RNA virus L proteins, abolished activity of other NNS RNA virus L proteins (107). Furthermore, an –LDD– motif is present at positions 1095–1097. Similar motifs with alterations in the first amino acid have been described and discussed as active sites for some RNA-dependent RNA polymerases of plant, animals, and bacterial viruses (108–111). Even though transcriptase and replicase activities have not yet been demonstrated, the L protein is regarded as an RNA-dependent RNA polymerase (Fig 4B; Table IV).

8. *Nonstructural Proteins*

A nonstructural glycoprotein has recently been discovered in EBO (69, 112). This protein, designated sGP, is expressed from the glycoprotein gene by RNA editing (Fig. 8). sGP shares about 300 N-terminal amino acids with GP but has a different C terminus (~70 amino acids), which contains many charged residues as well as conserved cysteines. The protein is directed into the endoplasmic reticulum and becomes *N*- and *O*-glycosylated. sGP is secreted into culture medium in high quantities. The function of this nonstructural protein is unknown, but it could modulate the host immune response by binding antibodies. No similar protein is found in MBG (Table IV).

MBG isolates, on the other hand, possess a second small open reading frame (ORF) located within the ORF of gene 2 (65). Expression would result in an extremely basic protein of 60 amino acids. The putative product shares features with a recently identified nonstructural protein of vesicular stomatitis virus (VSV). This protein is also encoded by a second ORF of gene 2, which normally encodes for the P protein of VSV (113). The overlapping ORF in gene 2 is not found in EBO.

D. Virus Replication

1. *Virus Growth in Cell Cultures*

Vero cells, especially clone E6, are the most widely used line for virus isolation and propagation. Primary virus isolation has also been successful in MA-104 and SW13 cells (30, 114). Furthermore, a variety of other cell lines have been tested as substrates for filovirus replication (41, 114, 115). This includes a recently developed human microvascular endothelial cell line (HMEC-1) (H. Feldmann, unpublished data), primary cultures of human umbilical cord vein endothelial cells (HUVEC) (116), and human peripheral blood monocytes/macrophages (117).

MBG and EBO subtype Zaire cause lytic infections in cell culture with distinct cytopathogenic effects. Infection with EBO subtypes Sudan and Reston proceeds more slowly, and cytopathogenic effects are not as obvious. The course of filovirus infection in tissue culture can be controlled by indirect immunofluorescence assay (IFA) using antibodies directed against viral antigens. Plaque assays are performed in Vero E6 cells; however, the ratio of plaque-forming units to infectious particles is thought to be relatively low. The number of infectious particles may therefore be underestimated in this assay. When there are few or no cytopathogenic effects, reverse transcriptase–polymerase chain reaction (RT-PCR) on viral RNA isolated from infected cells and tissue

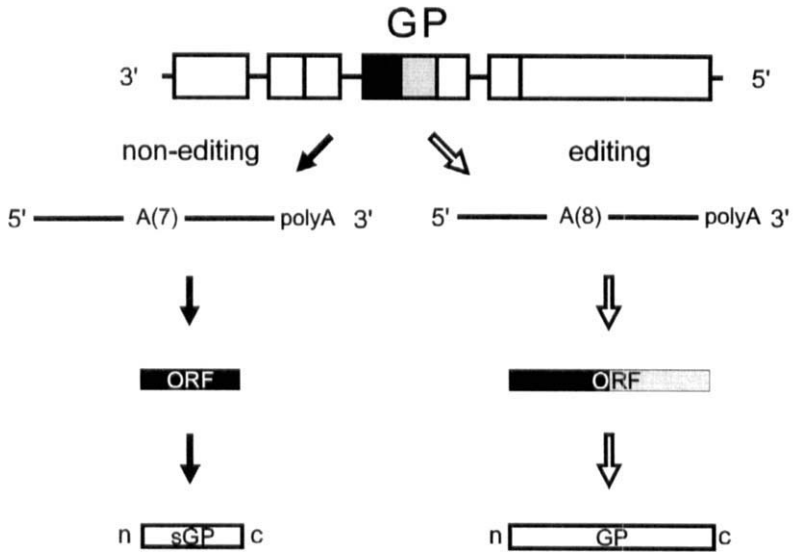

FIG. 8. Expression strategies of gene 4 of type Ebola viruses. Gene 4 of Ebola viruses is transcribed from two open reading frames. The primary gene product is a small glycoprotein (sGP). Full-length glycoprotein (GP) is expressed by RNA editing. *Key:* A, adenosine residue; c, carboxy-terminal end of proteins; n, amino-terminal end of proteins; GP, glycoprotein gene, 3′ and 5′, terminal ends of genomes and subgenomic RNAs.

culture supernatants can be helpful for quantification (116, 118). Furthermore, Northern blot hybridization and/or analysis of *in vivo* labeled viral subgenomic RNA have been used to study the course of infection (83, 119).

Viral RNA synthesis in tissue culture is detectable by at least 7 hr postinfection, reaches a maximum by 18 hr, and declines thereafter. Cytopathogenic effects are not seen before 48 hr postinfection. The first mRNA to be detected is NP-specific; it reaches levels sufficient to produce protein by 7 hr postinfection (119). This is in line with other NNS-RNA viruses, showing transcription to start at the most 3′-located gene of the linear genome (120, 121). All proteins are detectable by *in vitro* translation of polyadenylated RNA isolated 18 hr postinfection; thereafter the yield of translation products decreases (119). Recently, a PCR assay detected genomic RNA of MBG Strain Musoke (MUS) particles in clarified supernatants of infected cells as early as 12 hr postinfection, indicating that the replication cycle is approximately 12 hr (116). More sensitive technologies such as PCR have to be used in order to evaluate precisly the course of transcription and replication for filoviruses.

2. *Virus Entry*

Cell entry seems to be mediated by the GP as the only surface protein of virion particles. Studies on MBG MUS infections of hepatocytes have identified the asialoglycoprotein receptor present on these cells as a receptor candidate (122). However, one has to postulate additional receptors, since the asialoglycoprotein receptor is not expressed on many virus-susceptible cells. Furthermore, MBG GP is not generally lacking sialic acid, and sialylation has been shown to be cell-line-dependent (68). Whether the next step in virus entry involves a fusion process at the plasma membrane or fusion following endocytosis of virus particles is not known. The uncoating mechanism has not been studied either.

3. *Transcription, Translation, and Genome Replication*

Filovirus transcription and replication take place in the cytoplasm of infected cells. The data available so far suggest that the mechanisms involved resemble those observed for other NNS RNA viruses (Fig. 5). Transcription probably starts with a short (+)-leader sequence as in the case of some other NNS RNA viruses (123, 124). Subsequently, genomes are transcribed into monocistronic subgenomic RNA (mRNA) species which are complementary to viral genomic RNA and polyadenylated. Seven subgenomic RNA species have been detected in filovirus-infected cells by either Northern blot hybridization or RT-PCR amplification. There is no evidence for larger amounts of bi- or multicistronic subgenomic RNA species (72, 83, 119). Recent data on RNA amplification and direct sequencing of amplified products of four (NP, VP35, VP40, VP30) of the seven polyadenylated subgenomic RNA species of MBG MUS (125) revealed that the 5′ ends of the transcripts are two bases shorter than previously published (71, 72, 83). Thus, it appears that all start signals of filovirus genes possess the consensus sequence 3′–CUNCNUNUAAUU–5′ (Fig. 5). The 3′ ends of the transcripts carry a poly(A) tail generated by a stuttering mechanism of the viral polymerase at a run of five or six uridine residues located at the 5′ end of all transcription stop signals (72, 83). Therefore, the sequence 3′–UAAUUCUUUUU(U)–5′ serves as a transcription stop and polyadenylation signal (Fig. 5). As mentioned above, both signals carry the pentamer 3′–UAAUU–5′, which is characteristic for the family and a unique feature among NNS RNA viruses. The function of the pentamer is unknown, but it could serve as the recognition site for the polymerase complex. The surrounding semiconserved sequences may then mediate the exact initiation of transcription and termination–polyadenylation events.

Filovirus transcripts contain unusually long untranslated regions, especially at the 3′ ends. The 5′ end untranslated regions show a potential for formation of stable hairpin structures, which might play a role in transcript stability and ribosome binding (72). With the exception of the L protein, *in vitro* translation of purified subgenomic RNAs resulted in products comigrating on SDS-PAGE with the corresponding structural proteins. The finding that the L protein cannot be translated *in vitro* is probably the result of the low copy number of the L transcript, which has only been detected by RNA amplification (72, 83). The role of gene overlaps in regulation of transcription is unknown. Sanchez *et al.* (72) proposed that the downstream start site may allow the polymerase to recognize it after mRNA polyadenylation and possibly to reposition itself to initiate transcription. This "back up" mechanism is supported by the finding that attenuation of filovirus genes with start sites in overlaps does not occur to any greater degree than has been discussed for a much larger overlap found in the respiratory syncytial virus genome (126). Alternatively, the polymerase may occasionally terminate transcription at the overlap and initiate transcription of the downstream gene without polyadenylation of the upstream gene, but there is no evidence for detectable levels of transcripts lacking poly(A) tails.

With the EBO GP gene, transcription occurs from two open reading frames (Fig. 8). The primary gene product is a small nonstructural glycoprotein that is secreted from infected cells. Expression of full-length GP is achieved by transcriptional editing of a single nucleotide at a run of uridine residues (69, 112). MBG GP is expressed in a single frame and the gene does not contain sequences favoring RNA editing. A second ORF has been described for MBG MUS, but a corresponding gene product has not yet been identified (81). The difference in GP gene expression is one of several important distinctions between MBG and EBO (Table III).

The switch mechanism between transcription and replication is unknown. As with other NNS-RNA viruses, synthesis of the NP protein could be a key factor. Encapsidation and polymerase complex entry site are probably located on the 3′ leader sequence. The fact that the extremities of the genomes are complementary suggests a single identical encapsidation site on the genome and antigenome and an identical entry signal for the polymerase complex for both transcription and replication. Replication works via a full-length (+)-strand antigenome which serves as the template for synthesis of (–)-strand genome molecules. Encapsidated genomic RNA is incorporated into RNP. Those complexes may in part form inclusion bodies, which are prominent in infected cells (Fig. 5).

4. Virus Assembly and Exit

Virions usually bud at the plasma membrane, and the budding process is probably mediated at membrane locations where GP is incorporated. The cytoplasmic tail of GP is thought to interact with VP40 and/or VP24. VP40 may mediate the linkage between the RNP and the membrane proteins. Mature particles exit preferentially in a vertical mode, but budding via the longitudinal axis has also been observed. In macrophages, budding has also been observed at intracytoplasmic membranes surrounding vacuoles which form during infection (117). It is expected that the mechanism is similar to the situation at the plasma membrane.

IV. Clinical Virology

A. Clinical Syndrome

The onset of the disease is sudden, with fever, chills, headache, myalgia, and anorexia. This may be followed by symptoms such as abdominal pain, sore throat, nausea, vomiting, cough, arthralgia, diarrhea, and pharyngeal and conjunctival injection. Patients are dehydrated, apathetic, and disoriented and may develop a characteristic nonpruritic, maculopapular centripetal rash associated with varying degrees of erythema and then desquamate by day 5 or 7 of the illness. Hemorrhagic manifestations develop during the peak of the illness; they are of prognostic value for the disease. Bleeding into the gastrointestinal tract is most prominent along with petechiae and hemorrhages from puncture wounds and mucous membranes. Laboratory parameters are less characteristic, but the following findings are associated with the disease: leukopenia (as low as 1000/μl), left shift with atypical lymphocytes, thrombocytopenia (50,000–100,000/μl), markedly elevated serum transaminase levels (typically AST exceeding ALT), hyperproteinemia, and proteinuria. Prothrombin and partial thromboplastin times are prolonged and fibrin split products are detectable. In a later stage, secondary bacterial infection may lead to elevated white blood cell counts.

Nonfatal cases show fever for about 5–9 days; fatal cases develop clinical signs early during infection, and death commonly occurs between days 6 and 16 after the development of hemorrhage and hypovolemic shock (Fig. 9). Mortality is high for the African members of the family and varies between 22 and 88%, depending on the virus. The

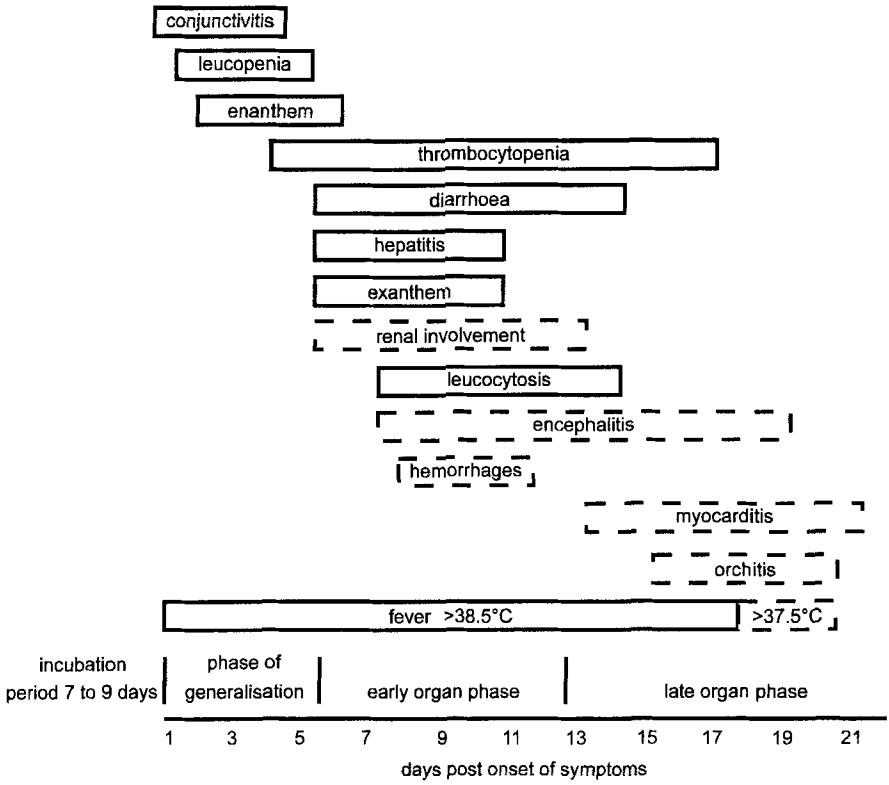

FIG. 9. Clinical course of hemorrhagic fever caused by Marburg virus. The drawing shows a summary of clinical observations during the 1967 outbreak of hemorrhagic fever caused by Marburg virus (adapted from W. Stille and E. Böhle [7]). *Key:* closed boxes, invariably present; open boxes, sometimes present.

highest rate has been reported for EBO Zaire. MBG infections are associated with the lowest mortality rates; however, most patients have been treated under European medical care standards, unlike in most of the EBO cases. The "Asian" filoviruses (EBO Reston) seem to possess a very low pathogenicity for humans or even to be apathogenic. This is interesting since genetic analyses have shown that EBO Reston seems to be most closely related to EBO Zaire (69).

Convalescence is prolonged and sometimes associated with myelitis, recurrent hepatitis, psychosis, or uveitis. An increased risk of abortion does exist for pregnant women, and clinical observations indicate a high death rate for children of infected mothers (20, 25, 127–131).

B. Diagnosis

Filoviruses cause acute infections in a variety of laboratory animals, although natural infections have only been reported in humans and nonhuman primates. In tropical settings, the identification of filoviral HF may be difficult since the most common causes of severe, acute febrile disease are malaria and typhoid fever. A wide range of infectious diseases has to be considered next, such as shigellosis, menigococcal septicemia, plague, leptospirosis, anthrax, relapsing fever, typhus, murine typhus, yellow fever, Chikungunya fever, Rift Valley fever, HF with renal syndrome, Crimean Congo HF, Lassa fever, and fulminant viral hepatitis. Travel, treatment in local hospitals, and contact with sick persons or wild and domestic monkeys are useful historical features in returning travelers, especially from Africa. Diagnosis of single cases is extremely difficult, but the occurrence of clusters of cases with prodromal fever followed by cases of hemorrhagic diatheses and person-to-person transmission are suggestive of viral HF, and containment procedures have to be initiated.

In filoviral HF, prostration, lethargy, wasting, and diarrhea seem to be more severe than observed in other viral HF patients. The rash is characteristic and extremely useful in differential diagnosis. Virologic diagnosis can be achieved during the febrile phase of the disease. Isolation attempts from serum and/or other clinical material should be performed using Vero or MA-104 cells (monkey kidney cells). However, most filoviruses do not cause extensive cytopathogenic effects on primary isolation. The most useful animal system after nonhuman primates is guinea pigs, which develop fever within 10 days of primary infection. Several passages, however, are necessary to produce a uniformly fatal disease. Often filoviruses do not kill newborn mice on primary isolation, suggesting that the most widely used animal system in laboratories may not be successful for virus isolation (30, 34, 128).

Laboratory diagnosis can be achieved in two different ways: measurement of the host-specific immunological response to the infection and detection of viral antigen and genomic RNA in the infected host (Table V). The most commonly used assay to detect antibodies to filoviruses is the indirect immunofluorescence assay (IFA) on acetone-fixed infected cells inactivated by gamma irradiation. The use of this assay, however, has been quite misleading, since a significant proportion of human and monkey sera will react with filovirus antigen without showing any symptoms of disease. Therefore, IFA results should be confirmed at least by an additional assay. Confirmatory tests include Western blot and enzyme-linked immunosorbent assays (ELISA). Direct IgG and

IgM ELISA are based on detergent-extracted infected cells adsorbed to plastic plates (55, 132, 133). In addition, an IgM capture assay has been developed which has correctly diagnosed acute infections with filoviruses in nonhuman primates, but still requires evaluation in humans (T. G. Ksiazek, personal communication). Radioimmune assays (RIA) are available but have not been evaluated for their use in diagnosis (70).

Direct detection of virus antigen, virus particles, and viral RNA can be achieved by several assays (Table V). Electron microscopy has been particularly useful in the diagnosis of filovirus infections (19, 30, 58, 134). Viral structures can be visualized by negative contrast electron microscopy after ultracentrifugation and fixation of initial passage cell culture supernatants. Thin-section microscopy can be performed on any infected material or infected cells which have been prepared by any standard fixation procedure. Immunohistochemisty on formalin-fixed material and paraffin-embedded tissues can be used for detection

TABLE V

LABORATORY DIAGNOSIS

Test	Target	Source	Remarks
Indirect immunofluorescence assay (IFA)	Antiviral antibodies	Serum	Simple to perform, but prone to nonspecific positives and *subjective interpretation*
Enzyme linked immunosorbent assay (ELISA)	Antiviral antibodies	Serum	Specific and sensitive, but initial response slower than IFA
Immuno blot	Antiviral antibodies	Serum	Protein-specific, but interpretation sometimes difficult
Antigen ELISA	*Viral antigen*	*Blood, serum,* tissues	*Rapid and sensitive, but* requires special equipment
Immunohistochemistry	Viral antigen	Tissues (*e.g.,* skin, liver)	Inactivated material, but requires time
Fluorescence assay (FA)	Viral antigen	Tissues (*e.g.,* liver)	Rapid and easy, but subjective information
Polymerase chain reaction (PCR)	Viral nucleic acid	Blood, serum, tissues	Rapid and sensitive, but requires expensive and special equipment
Electron microscopy	Viral particle	Blood, tissues	Unique morphology (immunostaining possible), but insensitive and requires expensive equipment
Virus isolation	Viral particle	Blood, tissues	Virus available for studies, but requires time

of filoviruses (30, 63), as can immunofluorescence on impression smears of tissues (135). Antigen detection ELISA (136) and reverse transcriptase–polymerase chain reaction (RT-PCR) (118) have been successfully used to detect filoviruses in clinical material. During the EBO Reston outbreak in 1989 both assays demonstrated their sensitivity and showed confirmation in nearly every case.

C. Patient Management and Prevention of Infection

A virus-specific treatment does not exist. Supportive therapy should be directed toward maintenance of effective blood volume and electrolyte balance. Management of shock, cerebral edema, renal failure, coagulation disorders, and secondary bacterial infection may be life-saving for patients. Heparin treatment should be considered only in cases with clear evidence of disseminated intravascular coagulopathy (DIC). Human interferon and human reconvalescence plasma have been used to treat patients in the past. Use of both therapies would be reasonable; however, there is no experimental data showing their efficacy. On the contrary, filoviruses are resistant to the antiviral effects of interferon, and interferon administration to monkeys has failed to increase survival rate or virus titer reduction. Ribavirin does not have any effect on filoviruses *in vitro* and thus is probably not of any clinical value, unlike in some other viral HFs. Isolation of patients is recommended, and protection of medical and nursing staff is required. This can be achieved by strict barrier nursing techniques and addition of HEPA filtered respirators for aerosol protection when feasible. For information regarding management of patients with suspected filoviral HF and approaches to minimize spread of virus in outbreak situations, especially in Africa, see published guidelines and recommendations (1, 34, 137–140).

Even though outbreaks of filovirus HF have been rare and mainly restricted to a small number of cases, vaccines would be of value for medical personnel in Africa as well as for laboratory personnel. Cross-protection among different EBO subtypes in experimental animal systems has been reported, suggesting the general value of vaccines (32, 141). Inactivated vaccines have been developed by formalin or heat treatment of cell-culture-propagated MBG and EBO subtypes Sudan and Zaire (142–144). Protection, however, has only been achieved by careful balance of the challenge dose and virulence. Because of the biohazardous nature of the agents, recombinant vaccines would be the way to go in the future. Immunizations of monkeys with purified NP and GP have demonstrated the induction of the humoral and cellular immune response and protected animals against challenge with lethal

doses (145). Thus, those two proteins and perhaps the sGP (EBO) may be candidates for recombinant vaccines. Recombinant GP-vaccinia- and baculoviruses have already been engineered for MBG MUS and EBO strain Mayinga (MAY), but have not yet been tested for a protective effect in animals (Centers for Disease Control and Prevention, Atlanta, Georgia, USA, unpublished data; Institute of Virology, Marburg, Germany, unpublished data; Institute of Molecular Biology, Koltsovo, Novosibirsk, Russian Federation).

Wild-caught monkeys are an important source for the introduction of filoviruses. This was clearly demonstrated in 1967 for MBG (127), in 1989, 1992, and 1996 for EBO Reston (30, 40, Centers for Disease Control and Prevention, personal communication), and in 1994 for the Ivory Coast EBO case (26). Quarantine of imported nonhuman primates and professional handling of animals will help prevent an introduction to humans. Guidelines for quarantine and proper handling of monkeys in medical research have been published (146).

Filovirus infectivity is quite stable at room temperature (20°C), but is destroyed in 30 min at 60°C. Infectivity is also destroyed by ultraviolet and gamma irradiation, formalin (1%), lipid solvents (deoxycholate, ether), β-propiolactone, and hypochloric and phenolic disinfectants (2, 138, 147).

V. Pathology and Immunology

A. Pathology in Experimental Animals

Monkeys, guinea pigs, suckling mice, and hamsters have been experimentally infected with filoviruses (30, 61, 114, 141, 148–150). MBG and EBO subtype Zaire are highly virulent for most of these species, and infection with low-passage virus stocks usually ends in death. Subtypes Sudan and Reston of EBO are less virulent, often causing a self-limited infection in guinea pigs and monkeys (32, 114, 151).

The incubation period for rhesus and African green monkeys inoculated with MBG and EBO subtype Zaire is 4–16 days. High titers of viruses can be detected in liver, spleen, lymph nodes, and lungs by onset of clinical symptoms. All of these organs, especially liver, show severe necrosis due to virus replication in parenchymal cells. Little inflammatory response at those sites is typical, which suggests that classical immunopathology may not be an important pathogenic consideration. Interstitial hemorrhage occurs and is most prominent in the gastrointestinal tract. In infected nonhuman primates, thrombocytopenia has been found accompanied by aggregation disorders of remain-

ing platelets in response to agonists such as ADP and collagen (61, 152). Histopathological damage of the target organs is at odds with serum transferase levels showing increase of ALT and AST with a ratio of AST:ALT of 7:1. This argues against hepatocellular dysfunction and raises the question of extrahepatic targets. Recent morphologic studies on EBO Reston-infected monkeys of the 1989 outbreak demonstrated extensive virus replication in tissue macrophages, interstitial fibroblasts of many organs, circulating monocytes/macrophages and, less frequently, in endothelial cells, hepatocytes, adrenal corticoid cells, and renal tubular epithelium (33). Similar results have been reported from experimentally infected MBG and EBO subtype Zaire monkeys (62). The pathophysiological basis for the hemorrhage and shock is still unknown. Prostaglandin-mediated dysfunction of endothelial cells and platelets and an unspecific immune response have been suggested to play an important role in the pathogenesis of the shock syndrome (152). Recent data, however, suggest that effects of viral infection on endothelial cells and/or virus-mediated release of mediators from infected monocytes/macrophages may be more important.

B. Pathology in Humans

In fatal cases, generalized hemorrhage is found macroscopically in most organ systems. Microscopic changes include focal necrosis in liver, lymphatic organs, kidneys, testes, and ovaries. The liver, while universally involved, with large eosinophilic intracytoplasmic inclusion bodies in hepatocytes and Councilman-like bodies within necrotic foci, is not the site of massive, potentially fatal necrosis. Generalized lymphoid necrosis is characteristic for the disease, and renal tubular necrosis is commonly found in agonal stages. A diffuse encephalitis as described for many viral infections has been observed in patients. Activation of the clotting system occurs and intravascular fibrin thrombi have been observed. Viral antigen can be detected by immunohistochemistry and electron microscopy in many organs, especially the liver, kidneys, spleen, and adrenal glands. Viral persistence has been demonstrated for MBG cases by isolation of virus from liver biopsy material and the anterior chamber of the eye after 4–5 weeks and semen after 12 weeks, despite an apparently normal immune response (20, 63, 127, 128, 134, 153).

C. Immunology

The mechanisms of recovery from filovirus infections in humans and wild as well as laboratory animals are unknown. *In vitro* neutralization has never been demonstrated by plaque reduction in cell culture

systems, and protection by convalescence sera has never been clearly shown. Fatal filovirus infections usually end with high viremia and no evidence of an immune response. In humans and monkeys they lead to extensive disruption of the parafollicular regions in the spleen and lymph nodes that contain the antigen-presenting dendritic cells (63). EBO Reston infection in monkeys is an exception in that a rise in nonproductive antibodies occurs shortly before death (128), Thus, cell-mediated immunity seems to mediate recovery from filovirus infections, although proof has not yet been presented.

GP is assumed to be the major antigenic molecule of virion particles. Its interaction with the host immune system may be modulated by the high content of carbohydrates. These sugars might cover antigenic epitopes, as has been demonstrated in other systems (154). For EBO, sGP production and secretion might interfere with the host immune response by neutralizing effective antibodies. As already mentioned filovirus GP molecules carry a presumably immunosuppressive domain close to the C terminus (Figs. 6A and 7). Peptides synthesized according to that 26-amino-acid-long region (Fig. 7) inhibited the blastogenesis of lymphocytes in response to mitogens, induced production of cytokines, and increased proliferation of mononuclear cells *in vitro.* Infected animals showed increased levels of mediators, in particular interferon and TNF. An activation of natural killer cells has been observed in the earlier stages of infection, whereas a complete lack of it has been observed in the later stages (155, 156). These findings are in line with the observation of immunosuppression in monkeys experimentally infected with filoviruses (MBG, EBO) and in humans and of proliferation of filoviruses in macrophages and monocytes *in vivo* and *in vitro* (33, 61–63, 117, 149). Monkeys that survived experimental MBG infection were susceptible to reinfection and showed shorter incubation periods and increased viremia (157). It is not yet known if the immunosuppressive domain on the GP is functional on mature molecules, but evidence for GP mediation of the above-mentioned effects has recently been reported (145).

D. Pathophysiology

The pathophysiological changes that make filovirus infections so devastating are just beginning to be unraveled. Pathogenesis in fatal infection in human and nonhuman primates is similar, suggesting the primate system as a model for studying filovirus HF (61, 62, 152, 158, 159). Clinical and biochemical findings support the anatomical observations of extensive liver involvement, renal damage, changes in vascular permeability including endothelial damage, and activation of the

clotting cascade. Visceral organ necrosis is a consequence of virus replication in parenchymal cells. However, no organ, not even the liver, shows sufficient damage to account for death. The role of disseminated intravascular coagulation (DIC) in pathogenesis is still controversial, since a laboratory confirmation of DIC in human infections has never been demonstrated. In nonhuman primates the intrinsic clotting pathway is most affected, whereas the extrinsic pathway is spared. The consequence is DIC in the final stages of the infection when parenchymal necrosis is extensive and leads to common terminal pathways, including DIC.

Laboratory parameters in the crucial early stage of filovirus HF, such as high AST:ALT ratio, normal bilirubin levels, and marked lymphopenia followed by a dramatic neutrophilia with left shift, suggest extrahepatic targets of infection. As with some other HFs [hemorrhagic fever with renal syndrome (HFRS), dengue HF, Lassa fever], fluid distribution problems and platelet abnormalities are dominant clinical manifestations indicating dysfunction or damage of endothelial cells and platelets. Post mortem there is little monocyte or macrophage infiltration in sites of parenchymal necrosis, suggesting that dysfunction of white blood cells also occurs. Morphological studies on EBO Reston-infected monkeys from the 1989 epizootic (33) and monkeys experimentally infected with EBO Zaire (62) showed that monocytes/macrophages and fibroblasts may be the preferred sites of virus replication in early stages, whereas other cell types may become involved as the disease progresses. Human monocytes/macrophages in culture are also sensitive to infection resulting in massive production of infectious virus and cell lysis (117). Although the studies on infected nonhuman primates did not identify endothelial cells as sites of massive virus replication, *in vitro* studies and post-mortem observations of human cases clearly demonstrated that endothelial cells of human origin are suitable targets for virus replication (63, 116). Here infection leads to cell lysis, indicating that damage of endothelial cells may be an important pathophysiological parameter during infection.

Besides evidence for direct vascular involvement in infected hosts, the role of active mediator molecules in the pathogenesis of the disorders has to be discussed. Although the source of these mediators during filovirus infections is still unknown, candidate cells exist. Besides the endothelium, which has yet to be examined for production and secretion of such mediators, the common denominator remains the monocyte/macrophage, a cell type shown to be a site of virus replication *in vivo* (33, 62, 63) and *in vitro* (117). Monocytes/macrophages are known as a pivotal source of different proteases, H_2O_2, and mediators such as tumor necrosis factor alpha (TNF-α), interleukins 6 and 8, and

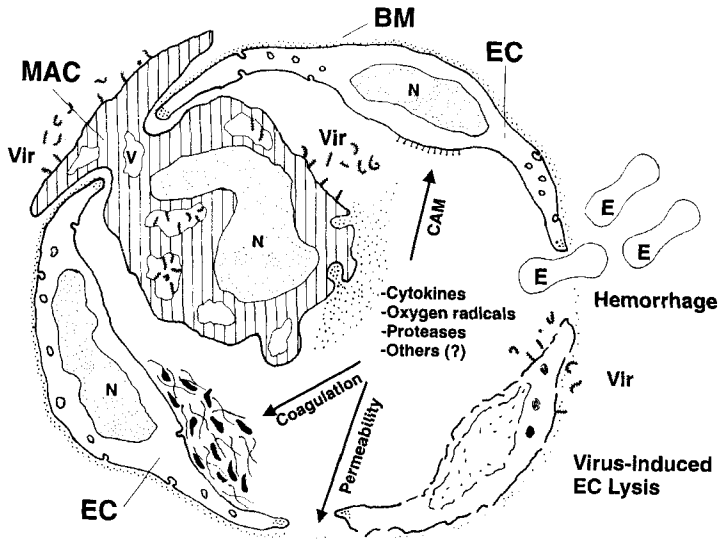

FIG. 10. Schematic drawing illustrating the possible role of macrophages and endothelial cells in the development of hemorrhagic fever caused by filoviruses. EC, endothelial cell; MAC, macrophage; Vir, virus particle; CAM, cell adhesion molecule; E, erythrocyte; BM, basement membrane; N, nucleus; V, vacuole.

platelet-derived growth factor (PDGF). TNF-α can result in secondary activation of mediators with important protective as well as deleterious effects. Recently it has been demonstrated that supernatants of filovirus-infected monocyte/macrophage cultures are capable of increasing paraendothelial permeability in an *in vitro* model (117). Examination for mediators in those supernatants revealed increased levels of secreted TNF-α, the prototype cytokine of macrophages. These data support the concept of a mediator-induced vascular instability and thus increased permeability as a key mechanism for the development of the shock syndrome seen in severe and fatal cases. Thus, the syndrome may be comparable to symptomatic shock in response to various endogenous and exogenous mediators (160–163). The bleeding tendency could be due to endothelial damage caused directly by virus replication as well as indirectly by cytokine-mediated processes. The onset of the bleeding tendency is supported by the loss of the integrity of the endothelium as demonstrated in tissue and organ culture (116) and infected animals (152). The hemorrhage occurs later in infection and could be due to extended damage which cannot be repaired by wound-healing mechanisms (164). The bleeding tendency is reinforced by a decrease in the bloodstream as a common consequence of shock.

The combination of viral replication in endothelial cells and virus-induced cytokine release from monocytes/macrophages may also promote a distinct proinflammatory endothelial phenotype that then triggers the coagulation cascade. A model summarizing the above discussed pathophysiological events is illustrated in Fig. 10.

NOTE

GenBank/EMBL data library accession numbers for nucleotide and amino acid sequences are as follows: MBG strain Musoke (EMBL data library, Z12132); MBG strain Popp (EMBL data library—X64405, X64406, X68493, X68494, X68495, Z29337); Ebola subtype Zaire, strain Mayinga (GenBank, L11365).

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REFERENCES

1. World Health Organization (1985). "Arthropod-Borne and Rodent-Borne Viral Diseases." WHO Technical Report Series 719.
2. Centers for Disease Control and Prevention (1993). "Biosafety in Microbiology and Biomedical Laboratories." US Department of Health and Human Services (HHS), Publication No. (CDC) 93-8395. US Government Printing Office, Washington, DC.
3. Holland, J. J. (1993). Genetic diversity of RNA viruses *Curr. Top. Microbiol. Immunol.* **176**, 1–226.
4. Peters, C. J., Sanchez, A., Feldmann, H., Rollin, P. E., Nichol, S. T., and Ksiazek, T. G. (1994). Filoviruses as emerging pathogens. *Semin. in Virol.* **5**, 147–154.
5. Martini, G. A., Knauff, H. G. Schmidt, H. A., Mayer, G., and Baltzer, G. (1968). Über eine bisher unbekannte, von Affen eingeschleppte Infektionskrankheit: Marburg-Virus-Krankheit. *Dtsch. Med. Wochenschr. 93*, 559–571.

6. Martini, G. A. (1971). Clinical Syndrome. *In* "Marburg Virus Disease" (G. A. Martini and R. Siegert, eds.), 1st Ed., pp. 1–9. Springer-Verlag, New York.
7. Stille, W., and Böhle, E. (1971). Clinical course and prognosis of Marburg virus ("green monkey") disease. *In* "Marburg Virus Disease" (G. A. Martini and R. Siegert, eds.), 1st Ed., pp. 10–18. Springer-Verlag, New York.
8. Stille, W., Böhle, E., Helm, E., vanRey, W., and Siede, W. (1968). Über eine durch *Cercopithecus aethiops* übertragene Infektionskrankheit. *Dtsch. Med. Wochenschr.* **93**, 572–582.
9. Stojkovic, L. J., Bordjoski, M., Gligic, A., and Stefanovic, Z. (1971). Two cases of Cercopithecus-monkeys-associated hemorrhagic fever. *In* "Marburg Virus Disease" (G. A. Martini and R. Siegert, eds.), 1st Ed., pp. 24–33. Springer-Verlag, New York.
10. Hennessen, W. (1971). Epidemiology of "Marburg virus" disease. *In* "Marburg Virus Disease" (G. A. Martini and R. Siegert, eds.), 1st Ed., pp. 161–165. Springer-Verlag, New York.
11. Haas, R., and Maass, G. (1971). Experimental infection of monkeys with the Marburg virus. *In* "Marburg Virus Disease" (G. A. Martini and R. Siegert, eds.), 1st Ed., pp. 136–143. Springer-Verlag, New York.
12. Gear, J. S. S., Cassel, G. A., Gear, A. J., Trappler, B., Clausen, L., Meyers, A. M., Kew, M. C., Bothwell, T. H., Sher, R., Miller, G. B., Schneider, J., Koornhoff, H. J., Comperts, E. D., Isaäcson, M., and Gear, J. H. S. (1975). Outbreak of Marburg virus disease in Johannesburg. *Br. Med. J.* **4**, 489–493.
13. Smith, D. H., Johnson, B. K., Isaäcson, M., Swanepoel, R., Johnson, K. M., Kiley, M. P., Bagshawe, A., Siongok, T., and Keruga, W. K. (1982). Marburg-virus disease in Kenya. *Lancet* **1**, 816–820.
14. Bowen, E. T. W., Lloyd, G., Harris, W. J., Platt, G. S., Baskerville, A., and Vella, E. E. (1977). Viral haemorrhagic fever in southern Sudan and Northern Zaire. *Lancet* **1**, 571–573.
15. Johnson, K. M., Webb, P. A., Lange, J. V., and Murphy, F. A. (1977). Isolation and partial characterization of a new virus causing acute haemorrhagic fever in Zaire. *Lancet* **1**, 569–571.
16. Babiker, Mohd el Tahir (1978). The haemorrhagic fever outbreak in Maridi, western Equatoria, southern Sudan. *In* "Ebola Virus Hemorrhagic Fever" (S. R. Pattyn, ed.), 1st Ed., pp. 125–127. Elsevier/North-Holland, Amsterdam.
17. Breman, J. G., Piot, P., Johnson, K. M., White, M. K., Mbuyi, M., Sureau, P., Heymann, D. L., van Nieuwenhove, S., McCormick, J. B., Ruppol, J. P., Kintoki, V., Isaäcson, M., van der Groen, G., Webb, P. A., and Ngvete, K. (1978). The epidemiology of Ebola haemorrhagic fever in Zaire, *1976*. *In* "Ebola Virus Hemorrhagic Fever" (S. R. Pattyn, ed.), 1st Ed., pp. 103–124. Elsevier/North-Holland, Amsterdam.
18. Francis, D. P., Smith, D. H., Highton, R. B., Simpson, D. I. H., Pacifiko Lolik, Isiaih Mayom Deng, Anthony Lagu Gillo, Ali Ahmed Idrtis, and Babiker El Tahir (1978). Ebola fever in the Sudan, 1976: Epidemiological aspects of the disease. *In* "Ebola Virus Hemorrhagic Fever" (S. R. Pattyn, ed.), 1st Ed., pp. 129–135. Elsevier/North-Holland, Amsterdam.
19. Murphy, F. A., van der Groen, G., Whitfield, S. G., and Lange, J. V. (1978). Ebola and Marburg virus morphology and taxonomy. *In* "Ebola Virus Hemorrhagic Fever" (S. R. Pattyn, ed.), 1st Ed., pp. 61–84. Elsevier/North-Holland, Amsterdam.
20. Pattyn, S. R., ed. (1978). "Ebola Virus Hemorrhagic Fever," 1st Ed., pp. 1–436. Elsevier/North-Holland, Amsterdam.
21. Smith, D. H., Francis, D., Simpson, D. I. H., and Highton, R. B. (1978). The Nzara outbreak of haemorrhagic fever. *In* "Ebola Virus Hemorrhagic Fever" (S. R. Pattyn,

ed.), 1st Ed., pp. 137–141. Elsevier/North-Holland, Amsterdam.
22. World Health Organization (1978). Ebola hemorrhagic fever in Sudan, 1976. *Bull. WHO* **56**, 247–270.
23. World Health Organization (1978). Ebola hemorrhagic fever in Zaire, 1976. *Bull. WHO* **56**, 271–293.
24. World Health Organization (1979). Viral hemorrhagic fever surveillance. *Weekly Epidemiol. Rec.* **54**, 342–343.
25. Baron, R. C., McCormick, J. B., and Zubeir, O. A. (1983). Ebola hemorrhagic fever in southern Sudan: Hospital dissemination and intrafamilial spread. *Bull. WHO* **61**, 997–1003.
26. LeGuenno, B., Formentry, P., Wyers, M., Gounon, P., Walker, F., and Boesch, C. (1995). Isolation and partial characterization of a new strain of Ebola virus. *Lancet* **345**, 1271–1274.
27. World Health Organization (1995a). Ebola hemorrhagic fever. *Weekly Epidemiol. Rec.* **70**, 149–152.
28. World Health Organization (1995b). Ebola hemorrhagic fever. *Weekly Epidemiol. Rec.* **70**, 241–242.
29. Centers for Disease Control and Prevention (1989). Ebola virus infection in imported primates—Virginia, 1989. *MMWR* **38**, 831–832, 837–838.
30. Jahrling, P. B., Geisbert, T. W., Galgard, D. W., Johnson, E. D., Ksiazek, T. G., Hall, W. C., and Peters, C. J. (1990). Preliminary report: Isolation of Ebola virus from monkeys imported to USA. *Lancet* **335**, 502–505.
31. Dalgard, D W., Hardy, R. J., Pearson, S. L., Pucak, G. J., Quander, R. V., Zack, P. M., Peters, C. J., and Jahrling, J. B. (1992). Combined simian hemorrhagic fever and Ebola virus infection in cynomolgus monkeys. *Lab. Animal Sci.* **42**, 152–157.
32. Fisher-Hoch, S. P., Brammer, L., Trappier, S. G., Hutwagner, L. C., Farrar, B. B., Ruo, S. L., Brown, B. G., Hermann, L. M., Perez-Oronoz, G. I., Goldsmith, C. S., Hanes, M. A., and McCormick, J. B. (1992). Pathogenic potential of filoviruses: Role of geographic origin of primate host and virus strain. *J. Infect. Dis.* **166**, 753–763.
33. Geisbert, T. W., Jahrling, P. B., Hanes, M. A., and Zack, P. M. (1992). Association of Ebola-related Reston virus particles and antigen with tissue lesions of monkeys imported to the United Staes. *J. Comp. Path.* **106**, 137–152.
34. Peters, C. J., Johnson, E. D., and McKee, K. T. (1991). Filoviruses and management and viral hemorrhagic fevers. *In* "Textbook of Human Virology" (R. B. Belshe, ed.), pp. 699–712. Mosby Year Book, St. Louis.
35. Peters, C. J., Johnson, E. D., Jahrling, P. B., Ksiazek, T. G., Rollin, P. E., White, J., Hall, W., Trotter, R., and Jaax, N. (1993). Filoviruses. *In* "Emerging Viruses" (S. S. Morse, ed.), pp. 159–175. Oxford University Press, Oxford.
36. Hayes, C. G., Burans, J. P., Ksiazek, T. G., DelRosario, R. A., Miranda, M. E. G., Manaloto, C. R., Barrientos, A. B., Robles, C. G., Dayrit, M. M., and Peters, C. J. (1992). Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. *Am. J. Trop. Med. Hyg.* **46**, 664–671.
37. Centers for Disease Control and Prevention (1990). Update: Filovirus infection in animal handlers. *MMWR* **39**, 221.
38. Centers for Disease Control and Prevention (1990). Update: Evidence of filovirus infection in an animal caretaker in a research/service facility. *MMWR* **39**, 296–297.
39. Centers for Disease Control and Prevention (1990). Update: Filovirus infection associated with contact with nonhuman primates or their tissues. *MMWR* **39**, 404–405.
40. World Health Organization (1992). Viral hemorrhagic fever in imported monkeys. *Weekly Epidemiol. Rec.* **67**, 142–143.

41. van der Groen, G., Johnson, K. M., Webb, P. A., Wulff, H., and Lange, J. (1978). Results of Ebola antibody survey in various population groups. In "Ebola Virus Hemorrhagic Fever" (S. R. Pattyn, ed.), 1st Ed., pp. 203–208. El Elsevier/North-Holland, Amsterdam.
42. Ivanoff, B., Duquesnoy, P., Languillat, G., Saluzzo, J. F., Georges, A., Gonzales, J. P., and McCormick, J. B. (1982). Haemorrhagic fever in Gabon. 1. Incidence of Lassa, Ebola and Marburg virus in Haut-Ogooue. *Trans. Soc. Trop. Med. Hyg.* **76**, 719–720.
43. Blackburn, N. K., Searle, L., and Taylor, P. (1982). Viral haemorrhagic fever antibodies in Zimbabwe school children. *Trans. Soc. Trop. Med. Hyg.* **76**, 803–805.
44. Bauree, P., and Bergmann, J. F. (1983). Ebola virus infection in man: A serological and epidemiological survey in the Cameroon. *Am. J. Trop. Med. Hyg.* **32**, 1465–1466.
45. Slenczka, W., Rietschel, M., Hoffmann, C., and Sixl, W. (1984). Seroepidemiologische Untersuchungen über das Vorkommen von Antikörpern gegen Marburg- und Ebola-Virus in Afrika. *Mitt. Oesterr. Ges. Tropenmed. Parasitol.* **6**, 53–60.
46. Johnson, B. K., Ocheng, D., Oogo, S., Gitau, L. G., Wambui, C., Gichogo, A., Libondo, D., Tukei, P. M., and Johnson, E. D. (1986). Seasonal variation in antibodies against Ebola virus in Kenyan fever patients. *Lancet* **1**, 1160.
47. van der Waals, F. J., Pomerov, K. L., Goudsmit, J., Asher, D. M., and Gajdusek, D. C. (1986). Hemorrhagic fever virus infection in an isolated rainforest area of central Liberia. Limitations of the indirect immunofluorescence slide test for antibody screening in Africa. *Trop. Geogr. Med.* **38**, 209–214.
48. Tomori, O., Fabiyi, A., and Sorungbe, A. (1988). Viral hemorrhagic fever antibodies in Nigerian population. *Am. J. Trop. Med. Hyg.* **38**, 407–410.
49. Gonzales, J. P., Johnson, E. D., Josse, R., Merlin, M., Georges, A. J., Abandja, J., Danyod, M., Delaporte, E., Dupont, A., Ghogomu, A., Kouka-Bemba, D., Madelon, M. C., Simav A., and Meunier, D. M. Y. (1989). Antibody prevalence against hemorrhagic fever viruses in randomized representative Central African populations. *Res. Virol.* **140**, 319–331.
50. Mathiot, C. C., Fontenille, D., Georges, A. J., and Coulanges, P. (1989). Antibodies to haemorrhagic fever viruses in Madagascar. *Trans. Soc. Trop. Med. Hyg.* **83**, 407–409.
51. Johnson, E. D., Gonzales, J. P., and Georges, A. (1993). Haemorrhagic fever virus activity in equatorial Africa: Distribution and prevalence of filovirus reactive antibodies in the Central African Republic. *Trans. Soc. Trop. Med. Hyg.* **87**, 530–535.
52. Johnson, E. D., Gonzales, J. P., and Georges, A. (1993). Filovirus activity among selected ethnic groups inhabiting the tropical rain forest of equatorial Africa. *Trans. Soc. of Trop. Med. Hyg.* **87**, 536–538.
53. Jahrling, P. B. (1995). Filoviruses and arenaviruses. *In* "Manual of Clinical Microbiology" (P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. M. Yolken, eds.), pp. 1068–1081. American Society for Microbiology, Washington, DC.
54. Centers for Disease Control and Prevention (1990). Update: Filovirus infection among persons with occupational exposure to nonhuman primates. *MMWR* **39**, 266–267, 273.
55. Becker, S., Feldmann, H., Will, C., and Slenczka, W. (1992). Evidence for occurrence of filovirus antibodies in humans and imported monkeys: Do subclinical filovirus infections occur worldwide? *Med. Microbiol. Immunol.* **181**, 43–55.
56. Henderson, B. E., Kissling, R. E., Williams, M. C., Kafuko, G. W., and Martin, M. (1971). Epidemiological studies in Uganda relating to the "Marburg" agent. *In* "Marburg Virus Disease" (G. A. Martini and R. Siegert, eds.), 1st Ed., pp. 166–176. Springer-Verlag, New York.
57. Fisher-Hoch, S. P., Perez-Oronoz, G. I., Jackson, E. L., Hermann, L. M., and Brown,

B. G. (1992). Filovirus clearance in non-human primates. *Lancet* **340**, 451–453.
58. Siegert, R., Shu, H.-L., Slenczka, W., Peters, D., and Müller, G. (1967). Zur Äthiologie einer unbekannten von Affen ausgegangenen Infektionskrankheit. *Dtsch. Med. Wochenschr.* **92**, 2341–2343.
59. Pokhodyaeu, V. A., Gonchar, N. I., and Pshenichnov, V. A. (1991). Experimental study of Marburg virus contact transmission. *Voprosy Virusologii* **36**, 506–508.
60. Bazhutin, N. B., Belanov, E. F., Spiridonov, V. A., Voitenko, A. V., Krivenchuk, N. A., Krotovf, S. A., Omelchenko, N. I., Tereschenko, A. Y., and Khomichev, V. V. (1992). The influence of the methods of experimental infection with Marburg virus on the features of the disease process in green monkeys. *Voprosy Virusologii* **37**, 153–156.
61. Murphy, F. A., Simpson, D. I. H., Whitfield, S. G., Zlotnik, I., and Carter, G. B. (1971). Marburg virus infection in monkeys. *Lab. Invest.* **24**, 279–291.
62. Ryabchikova, E. I., Kolesnikova, L. V., Tkachev, V. K., Pereboeva, L. A., Baranova, S. G., and Rassadkin, J. N. (1994). Ebola infection in four monkey species. Ninth International Conference on Negative Strand RNA Viruses, p. 164. Estoril, Portugal. [Abstract]
63. Zaki, S. (1995). Ebola virus infection. European Conference on Tropical Medicine, p. 2 (A22). Hamburg, Germany. [Abstract]
64. Kiley, M. P., Bowen, E. T. W., Eddy, G. A., Isaäcson, M., Johnson, K. M., McCormick, J. B., Murphy, F. A., Pattyn, S. R., Peters, D., Prozesky, O. W., Regnery, R. L., Simpson, D. I. H., Slenczka, W., Sureau, P., van der Groen, G., Webb, P. A., and Wulff, H. (1982). Filoviridae: A taxonomic home for Marburg and Ebola viruses? *Intervirology* **18**, 24–32.
65. Ströher, U., Sanchez, A., Klenk, H.-D., and Feldmann, H. (1995). The Marburg group of filoviruses: Genetic variability and characterization of a second ORF encoding a potential nonstructural protein. First European Meeting of Virology, P2/42. Würzburg, Germany. [Abstract]
66. Kiley, M. P., Cox, N. J., Elliott, L. H., Sanchez, A., DeFries, R., Buchmeier, M. J., Richman, D. D., and McCormick, J. B. (1988). Physicochemical properties of Marburg virus: Evidence for three distinct virus strains and their relationship to Ebola virus. *J. Gen. Virol.* **69**, 1957–1967.
67. Feldmann, H., Klenk, H.-D., and Sanchez, A. (1993). Molecular biology and evolution of filoviruses. *Arch. Virol.*, Supplement **7**, 81–100.
68. Feldmann, H., Nichol, S. T., Klenk, H.-D., Peters, C. J., and Sanchez, A. (1994). Characterization of filoviruses based on differences in structure and antigenicity of the virion glycoprotein. *Virology* **199**, 469–473.
69. Sanchez, A., Trappier, S., Mahy, B. W. J., Peters, C. J., and Nichol, S. T. (1996). The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3602–3607.
70. Richman, D. D., Cleveland, P. H., McCormick, J. B., and Johnson, K. M. (1983). Antigenic analysis of strains of Ebola viruses: Identification of two Ebola virus subtypes. *J. Infect. Dis.* **147**, 268–271.
71. Sanchez, A., Kiley, M. P., Klenk, H.-D., and Feldmann, H. (1992). Sequence analysis of the Marburg virus nucleoprotein gene: Comparison to Ebola virus and other non-segmented negative-strand RNA viruses. *J. Gen. Virol.* **74**, 347–357.
72. Sanchez, A., Kiley, M. P., Holloway, B. P., and Auperin, D. D. (1993). Sequence analysis of the Ebola virus genome: Organization, genetic elements, and comparison with the genome of Marburg virus. *Virus Res.* **29**, 215–240.
73. Bukreyev, A. A., Volchkov, V. E., Blinov, V. M., and Netesov, S. V. (1993). The VP35

and VP40 proteins of filoviruses: Homology between Marburg and Ebola viruses. *FEBS Lett.* **322**, 41–46.
74. Buchmeier, M. J., DeFries, R., McCormick, J. B., and Kiley, M. P. (1983). Comparative analysis of the structural polypeptides of Ebola virus from Sudan and Zaire. *J. Infect. Dis.* **147**, 276–281.
75. Cox, N. J., McCormick, J. B., Johnson, K. M., and Kiley, M. P. (1983). Evidence for two subtypes of Ebola virus based on oligonucleotide mapping of RNA. *J. Infect. Dis.* **147**, 272–275.
76. ICTV (1991). The order *Mononegavirales.* Paramyxovirus Study Group of the Vertebrate Subcommittee. Virology Division News. *Arch. Virol.* **117**, 137–140.
77. Smith, C. E. G., Simpson, D. I. H., and Bowen, E. T. W. (1967). Fatal human disease from vervet monkeys. *Lancet* **2**, 1119–1121.
78. Kissling, R. E., Robinson, R. Q., Murphy, F. A., and Whitfield, F. G. (1968). Agent of disease contracted from green monkeys. *Science* **160**, 888–890.
79. Peters, D., Müller, G., and Slenczka, W. (1971). Morphology, development, and classification of Marburg virus. *In* "Marburg virus disease" (G. A. Martini and R. Siegert, eds.), 1st Ed., pp. 68–83. Springer-Verlag, New York.
80. Regnery, R. L., Johnson, K. M., and Kiley, M. P. (1980). Virion nucleic acid of Ebola virus. *J. Virol.* **36**, 465–469.
81. Will, C., Mühlberger, E., Linder, D., Slenczka, W., Klenk, H.-D., and Feldmann, H. (1993). Marburg virus gene four encodes for the virion membrane protein, a type I transmembrane glycoprotein. *J. Virol.* **67**, 1203–1210.
82. Kiley, M. P., Regnery, R. L., and Johnson, K. M. (1980). Ebola virus: Identification of virion structural proteins. *J. Gen. Virol.* **49**, 333–341.
83. Feldmann, H., Mühlberger, E. Randolf, A., Will, C., Kiley, M. P., Sanchez, A., and Klenk, H.-D. (1992). Marburg virus, a filovirus: Messenger RNAs, gene order, and regulatory elements of the replication cycle. *Virus Res.* **24**, 1–19.
84. Bukreyev, A. A., Volchkov, V. E., Blinov, V. M., Dryga, S. A., and Netesov, S. V. (1995). The nucleotide sequence of the Popp (1967) strain of Marburg virus: A comparison with the Musoke (1980) strain. *Arch. Virol.* **140**, 1589–1600.
85. Volchkov, V. E., Blinov, V. M., Kotov, A. N., Chepurnov, A. A., and Netesov, S. V. (1993). The full-length nucleotide sequence of the Ebola virus. IXth International Congress of Virology, p. 299. Glasgow, Scotland. [Abstract]
86. Kiley, M. P., Wilusz, J., McCormick, J. B., and Keene, J. D. (1986). Conservation of the 3′ terminal nucleotide sequence of Ebola and Marburg viruses. *Virology* **149**, 251–254.
87. Sanchez, A., Kiley, M. P., Holloway, B. P., McCormick, J. B., and Auperin, D. D. (1989). The nucleoprotein gene of Ebola virus: Cloning, sequencing, and in vitro expression. *Virology* **170**, 81–91.
88. Elliott, L. H., Kiley, M. P., and McCormick, J. B. (1985). Descriptive analysis of Ebola virus proteins. *Virology* **147**, 169–176.
89. Elliott, L. H., Sanchez, A., Holloway, B. P., Kiley, M. P., and McCormick, J. B. (1993). Ebola protein analysis for the determination of genetic organization. *Arch. Virol.* **133**, 423–436.
90. Becker, S., Huppertz, S., Klenk, H.-D., and Feldmann, H. (1994). The nucleoprotein of Marburg virus is phosphorylated. *J. Gen. Virol.* **75**, 809–818.
91. Barr, J., Chamers, P., Pringle, C. R., and Easton, A. J. (1991). Sequence of the major nucleocapsid gene of pneumonia virus of mice: Sequence comparison suggests structural homology between nucleocapsid proteins of pneumoviruses, paramyxoviruses, rhabdoviruses and filoviruses. *J. Gen. Virol.* **72**, 677–685.

92. Morgan, E. M. (1991). Evolutionary relationships of paramyxoviruses nucleocapsid-associated proteins. *In* "The Paramyxoviruses" (D. W. Kingsbury, ed.), pp. 163–179. Plenum Press, New York.
93. Peeples, M. E. (1991). Paramyxovirus M proteins: Pulling it all together and putting it on the road. *In* "The Paramyxoviruses" (D. W. Kingsbury, ed.), pp. 427–456. Plenum Press, New York.
94. Banerjee, A. K. (1987). Transcription and replication of rhabdoviruses. *Microbiol. Rev.* **51**, 66–87.
95. Wagner, R. R. (1987). Rhabdovirus biology and infection: An overview. *In* "The Rhabdoviruses" (R. R. Wagner, ed.), pp. 9–74. Plenum Press, New York.
96. Volchkov, V. E., Blinov, V. M., and Netesov, S. V. (1992). The envelope glycoprotein of Ebola virus contains an immunosuppressive-like domain similar to oncogenic retroviruses. *FEBS Lett.* **305**, 181–184.
97. Bukreyev, A, A,, Volchkov V. E., Blinov, V. M., and Netesov, S. V. (1993). The GP-protein of Marburg virus contains the region similar to the "immunosuppressive domain" of oncogenic etroviruses P15E proteins. *FEBS Lett.* **323**, 183–187.
98. Feldmann, H., Will, C., Schikore, M., Slenczka, W., and Klenk, H.-D. (1991). Glycosylation and oligomerization of the spike protein of Marburg virus. *Virology* **182**, 353–356.
99. Geyer, H., Will, C., Feldmann, H., Klenk, H.-D., and Geyer, R. (1992). Carbohydrate structure of Marburg virus glycoprotein. *Glycobiology* **2**, 299–312.
100. Funke, C., Becker, S., Dartsch, H., Klenk, H.-D., and Mühlberger, E. (1995). Acylation of the Marburg virus glycoprotein. *Virology* **208**, 289–297.
101. Cianciolo, G. J., Copeland, T. J., Oroszlan, S., and Snyderman, R. (1985). Inhibition of lymphocyte proliferation by a synthetic peptide homologous to retroviral envelope proteins. *Science* **230**, 453–455.
102. Harris, D. T., Cianciolo, G. J., Snyderman, R., Argov, S., and Koren, H. R. (1987). Inhibition of human natural killer cell activity by a synthetic peptide homologous to a conserved region in the retroviral protein, p15E. *J. Immunol.* **138**, 889–894.
103. Kadota, J., Cianciolo, G. J., and Snyderman, R. (1991). A synthetic peptide homologous to retroviral transmembrane envelope proteins depressed protein kinase C-mediated lymphocyte proliferation and directly inactivated proteine kinase C: A potential mechanism for immunosuppression. *Microbiol. Immunol.* **35**, 443–459.
104. Volchkov, V. E., Chepurnov, A., Dryga, S., Becker, S., Blinov, V., Kotov, A., Ternovoj, V., Klenk, H.-D., and Netesov, S. V. (1994). Molecular characterization of a pathogenicity variant of Ebola virus. Ninth International Conference on Negative Strand RNA Viruses, p. 176. Estoril, Portugal. [Abstract]
105. Mühlberger, E., Sanchez, A., Randolf, A., Will, C., Kiley, M. P., Klenk, H.-D., and Feldmann, H. (1992). The nucleotide sequence of the L gene of Marburg virus, a filovirus: Homologies with paramyxoviruses and rhabdoviruses. *Virology* **187**, 534–547.
106. Barik, S. E., Rud, W., Luk, D., Banerjee, A. K., and Yong Kang, C. (1990). Nucleotide sequence analysis of the L gene of vesicular stomatitis virus (New Jersey serotype): Identification of conserved domains in L proteins of nonsegmented negative-strand RNA viruses. *Virology* **175**, 332–337.
107. Sleat, D. E., and Banerjee, A. K. (1993). Transcriptional activity and mutational analysis of recombinant vesicular stomatitis virus RNA polymerase. *J. Virol.* **67**, 1334–1339.
108. Kamer, G., and Argos, P. (1984). Primary structural comparison of RNA-dependent polymerases from plant, animal and bacterial viruses. *Nucleic Acids Res.* **12**, 7269–7282.

109. Wain-Hobson S., Sonigo, P., Danos, O., Cole, S., and Alizon, M. (1985). Nucleotide sequence of the AIDS virus, LAV. *Cell* **40**, 9–17.
110. Chiu I. M., Yaniv, A., Dahlberg, J. E, Gazit, A., Skunitz, S. F., Tronick, S. R., and Aaronson, S. A. (1985). Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses. *Nature (London)* **317**, 366–368.
111. Kemdirin, S., Palefsky, J., and Briedis, D. J. (1986). Influenza B virus PB1 protein: Nucleotide sequence of the genome RNA segment predicts a high degree of structural homology with the corresponding influenza A virus polymerase proteins. *Virology* **152**, 126–135.
112. Volchkov, V. E., Becker S., Volchkova, V. A., Ternovoj, V. A., Kotov, A. N., Netesov, S. V., and Klenk, H.-D. (1995). Gp mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. *Virology* **214**, 421–430.
113. Spiropoulou, C. F., and Nichol, S. T. (1993). A small highly basic protein is encoded in overlapping frame within the P gene of vesicular stomatitis virus. *J. Virol.* **67**, 3103–3110.
114. McCormick, J. B., Bauer, S. P., Elliott, L. H., Webb, P. A., and Johnson, K. M. (1983). Biological differences between strains of Ebola virus from Zaire and Sudan. *J. Infect. Dis.* **147**, 264–267.
115. Peters, C. J., Jahrling, P. B., Ksiazek, T. G., and Lupton, H. (1992). Filovirus contamination of cell cultures. *Develop. Biol. Standard* **76**, 267–274.
116. Schnittler, H. J., Mahner, F., Drenckhahn, D., Klenk, H.-D., and Feldmann, H. (1993). Replication of Marburg virus in human endothelial cells. A possible mechanism for the development of viral hemorrhagic disease. *J. Clin. Invest.* **91**, 1301–1309.
117. Feldmann, H., Bugany, H., Mahner, F., Klenk, H.-D., Drenckhahn, D., and Schnittler, H.-J. (1996). Filovirus-induced endothelial leakage triggered by infected monocytes/macrophages. *J. Virol.* **70**, 2208–2214.
118. Sanchez, A., and Feldmann, H. (1996). Detection of Marburg and Ebola virus infections by polymerase chain reaction assays. *In* "Frontiers in Virology—Diagnosis of Human Viruses by Polymerase Chain Reaction Technology" (Y. Becker and G. Darai, eds.), 2nd Ed., pp. 411–418. Springer-Verlag, New York.
119. Sanchez, A., and Kiley, M. P. (1987). Identification and analysis of Ebola virus messenger RNAs. *Virology* **157**, 414–420.
120. Kingsbury, D. W. (1974). The molecular biology of paramyxoviruses. *Med. Microbiol. Immunol.* **160**, 73–83.
121. Kolakofsky, D., Vidal, S., and Curran, J. (1991). Paramyxovirus RNA synthesis and P gene expression. *In* "The Paramyxoviruses" (D. W. Kingsbury, ed.), pp. 215–233. Plenum Press, New York.
122. Becker, S., Spiess, M., and Klenk, H.-D. (1995). The asialoglycoprotein receptor is a potential liver-specific receptor for Marburg virus. *J. Gen. Virol.* **76**, 393–399.
123. Colonno, R. J., and Banerjee A. K. (1977). Mapping and initiation studies on the leader RNA of vesicular stomatitis virus. *Virology* **77**, 260–268.
124. Leppert, M., Rittenhouse, L., Perrault, J., Summers, D. F., and Kolakofsky, D. (1979). Plus and minus strand leader RNAs in negative strand virus-infected cells. *Cells* **18**, 735–747.
125. Mühlberger, E., Trommer, S., Funke, C., Klenk, H.-D. and Becker, S. (1996). Determination of the termini of genomic RNA and mRNA species of Marburg virus. *Virology*, in press.
126. Collins, P. L. (1991). The molecular biology of human respiratory syncytial virus (RSV) of the genus *Pneumovirus*. *In* "The Paramyxoviruses" (D. W. Kingsbury, ed.), pp. 103–162. Plenum Press, New York.

127. Martini, G. A., and Siegert, R. (1971). "Marburg Virus Disease." 1st Ed. pp. 1–237. Springer-Verlag, New York.
128. Peters, C. J., Sanchez, A., Rollin, P. E., Ksiazek, T. G., and Murphy, F. A. (1996). Filoviridae: Marburg and Ebola viruses. *In* "Virology" (B. N. Fields, D. M. Knipe, and P. M. Howley, eds.), 3rd Ed., pp. 1161–1176. Raven Press, New York.
129. Wulff, H., and Conrad, J. L. (1977). Marburg virus disease. *In* "Comparative Diagnosis of Viral Diseases" (E. Kurstak, ed.), Vol. 2, pp. 3–33. Academic Press, New York.
130. Piot, P., Sureau, P., Bremen, G., Heyman, D., Kintoki, V., Masamba, M., Mbuyi, M., Miatudila, M., Ruppol, F., van-Nieuwenhove, S., White, M. K., van der Groen, G., Webb, P. A., Wulff, H., and Johnson, K. M. (1978). Clinical aspects of Ebola virus infection in Yambuku area, Zaire, 1976. *In* "Ebola Virus Haemorrhagic Fever" (S. R. Pattyn, ed.), pp. 7–14. Elsevier/ North-Holland, Amsterdam.
131. Sureau, P. H. (1989). Firsthand clinical observations of hemorrhagic manifestations in Ebola hemorrhagic fever in Zaire. *Rev. Infect. Dis.* **11**, 790–793.
132. Elliott, L. H., Bauer, S. P., Perez-Oronoz, G., and Lloyd, E. S. (1993). Improved specificity of testing methods for filovirus antibodies. *J. Virol. Methods.* **43**, 85–100.
133. Ksiazek, T. G. (1991). Laboratory diagnosis of filovirus infections in non-human primates. *Lab. Animal* **20**, 34–46.
134. Dietrich, M., Schumacher, H. H., Peters, D., and Knobloch, J. (1978). Human pathology of Ebola virus infection in Sudan. *In* "Ebola Virus" (S. R. Pattyn, ed.), pp. 37–42. Elsevier/North Holland, Amsterdam.
135. Rollin, P. E., Ksiazek, T. G., Jahrling, P. B., Hanes, M., and Peters C. J. (1990). Detection of Ebola-like viruses by immunofluorescence. *Lancet* **336(8730)**, 1591.
136. Ksiazek, T. G., Rollin, P. E., Jahrling, P. B., Johnson, E., Dalgard, D. W., and Peters, C. J. (1992). Enzyme immunosorbent assay for Ebola virus antigens in tissues of infected primates. *J. Clin. Microbiol.* **30**, 947–950.
137. Mitchell, S. W., and McCormick, J. B. (1982). "Mobile Clinical Laboratory Manual." pp. 1–60. Centers for Disease Control, Atlanta.
138. Centers for Disease Control and Prevention (1988). Management of patients with suspected viral hemorrhagic fever. *MMWR* **37 (Suppl 3)**, 1–16.
139. Foberg, U., Fryden, A., Isaksson, B., Jahrling, P. B., Johnson, A., McKee, K., Niklasson, B., Norrnann, B., Peters, C. J., and Bengtsson, M. (1991). Viral hemorrhagic fever in Sweden: Experiences from management of a case. *Scand. J. Infect. Dis.* **23**, 143–151.
140. World Health Organization (1995). Viral haemorrhagic fever—management of suspected cases. *Weekly Epidemiol. Rec.* **70**, 249–256.
141. Bowen, E. T. W., Platt, G. S., Lloyd, G., Raymond, R. T., and Simpson, D. I. H. (1980). A comparative study of strains of Ebola virus isolated from southern Sudan and northern Zaire in 1976. *J. Med. Virol.* **6**, 129–138.
142. Lupton, H. W., Lambert, R. D., and Bumgardner, D. L. (1980). Inactivated vaccine for Ebola virus efficacious in guinea pig model. *Lancet* **2**, 1294–1295.
143. Ignatyev, G. M., Agafonov, A. P., Strelysova, M. A., Kuzymin, V. A., Mainagasheva, G. I., Spirin, G. V., and Chernyi, N. B. (1991). A comparative study of the immunological indices in guinea pigs administered an inactivated Marburg virus. *Voprosy Virusologii* **36**, 421–423.
144. Agafonov, A. P., Ignatyev, G. M., Kuzymin, V. A., Akimenko, Z. L., Kosareva, T. V., and Kashentseva, E. A. (1992). The immunogenic properties of Marburg virus proteins. *Voprosy Virusologii* **37**, 58–61.
145. Agafanov, A. P., Ignatyev, G. M., Akimenko, Z. A., and Volchkov, V. E. (1993). Study of immunogenic and protective properties of Marburg virus GP, NP and VP40 proteins. IXth International Congress of Virology, p. 300. Glasgow, Scotland. [Abstract]

146. Centers for Disease Control and Prevention (1990). Update: Ebola-related filovirus infection in nonhuman primates and interim guidelines for handling nonhuman primates during transit and quarantine. *MMWR* **39(2)**, 22–24, 29–30.
147. Elliott, L. H., McCormick, J. B., and Johnson, K. M. (1982). Inactivation of Lassa, Marburg, and Ebola viruses by gamma irradiation. *J. Clin. Microbiol.* **16**, 704–708.
148. Kissling, R. E., Murphy, F. A., and Henderson, B. E. (1970). Marburg virus. *Ann. N. Y. Acad. Sci.* **174**, 932–945.
149. Baskerville, A., Fisher Hoch, S. P., Neild, G. H., and Dowsett, A. B. (1985). Ultrastructural pathology of experimental Ebola haemorrhagic fever virus infection. *J. Pathol.* **147**, 199–209.
150. Pereboeva, L. A., Tkachev, V. K., Kolesnikova, L. V., Krendeleva, L. I., and Ryabchikova, E. I. (1993). The ultrastructural changes in guinea pig organs during the serial passage of the Ebola virus. *Voprosy Virusologii* **38(4)**, 179–182.
151. Bowen, E. T. W., Lloyd, G., Harris, W. J., Platt, G. S., Baskerville, A., and Vella, E. E. (1977). Viral haemorrhagic fever in southern Sudan and northern Zaire. *Lancet* **1**, 571–573.
152. Fisher-Hoch, S. P., Platt, G. S., Neild, G. H., Southee, T., Baskerville, A., Raymond, R. T., Lloyd, G., and Simpson, D. I. H. (1985). Pathophysiology of shock and hemorrhage in a fulminating viral infection (Ebola). *J. Infect. Dis.* **152**, 887–894.
153. Murphy, F. A. (1978). Pathology of Ebola virus infection. *In* "Ebola Virus Haemorrhagic Fever" (S. R. Pattyn, ed.), pp. 37–42. Elsevier/North-Holland, Amsterdam.
154. Munk, K., Pritzer, E., Gutte, B., Garten, W., and Klenk, H.-D. (1992). Carbohydrate masking of an antigenic epitope of influenza virus haemagglutinin independent of the oligosaccharide size. *Glycobiology* **2**, 233–240.
155. Chepurnov, A. A., Ignatyev, G. M., and Volchkov, V. E. (1993). Immunologic and biochemical indices Ebola fever. IXth International Congress of Virology, p. 300. Glasgow, Scotland. [Abstract]
156. Ignatyev G. M., Streltsova, M. A., Agafonov, A. P., and Netesov, S. V. (1993). The study of immunological properties of inactivated Marburg virus. IXth International Congress of Virology, p. 300. Glasgow, Scotland. [Abstract]
157. Belanov, E. F., Bukreyev, A. A., Bazhutin, N. B., Spiridonov, V. A., Netesov, S. V., and Blinov, V. M. (1993). Monkeys *C. aethiops* are sensitive to repeated infection with Marburg virus. IXth International Congress of Virology, p. 299. Glasgow, Scotland. [Abstract]
158. Simpson, D. I. H., Zlotnik, I., and Rutter, D. A. (1968). Vervet monkey disease. Experimental infection of guinea pigs and monkeys with the causative agent. *Br. J. Exp. Pathol.* **49**, 458–464.
159. Ellis, D. S., Bowen, E. T. W., Simpson, D. I. H., *et al.* (1978). Ebola virus: A comparison, at ultrastructural level, of the behaviour of the Sudan and Zaire strains in monkeys. *Br. J. Exp. Pathol.* **59**, 584–593.
160. Majno, G., and Palade, G. (1961). Studies on inflammation I. The effect of histamine and serotonin on vascular permeability, an electron microscopic study. *J. Biophys. Biochem. Cytol.* **11**, 571–605.
161. Crone, C. (1987). The Malpighi lecture. From "porositates carnis" to cellular microcirculation. *Int. J. Microcic. Clin. Exp.* **6**, 101–122.
162. Michel, C. C. (1988). Capillary permeability and how it may change. *J. Physiol.* **404**, 1–29.
163. Schnittler, H.-J., Wilke, A., Gress, T., Sutton, N., and Drenckhahn, D. (1990). Role of actin and myosin in the control of paracellular permiability in pig, rat, and human vascular endothelium. *J. Physiol.* **431**, 379–401.
164. Wong, M. K. K., and Gotlieb, A. I. (1988). The reorganization of microfilaments

centrosomes and microtubules during in vitro small wound reendothelialization. *J. Cell. Biol.* **107**, 1777–1783.

165. Emond, R. T. D. (1978). Isolation, monitoring and treatment of a case of Ebola virus infection. *In* "Ebola Virus Hemorrhagic Fever" (S. R. Pattyn, ed.), 1st Ed., pp. 27–32. Elsevier/North-Holland, Amsterdam.
166. Heymann, D. L., Weisfeld, J. S., Webb, P. A., Johnson, K. M., Cairns, T., and Berquist, H. (1980). Ebola hemorrhagic fever: Tandala, Zaire, 1977–78. *J. Infect. Dis.* **142**, 372–376.
167. Johnson, K. M., Scribner, C. L., and McCormick, J. B. (1981). Ecology of Ebola virus: A first clue? *J. Infect. Dis.* **143**, 749–751.
168. Teepe, R. G. C., Johnson, B. K., Ocheng, D., Gichogo, A., Langatt, A., Ngindu, A., Kiley, M. P., Johnson, K. M., and McCormick, J. B. (1983). A probable case of Ebola virus hemorrhagic fever in Kenya. *East Afr. Med. J.* **60**, 718–722.
169. World Health Organization (1996). Outbreak of Ebola haemorrhagic fever in Gabon officially declared over. *Weekly Epidemiol. Rec.* **71**, 125–126.