An Introduction to Ebola: The Virus and the Disease

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Filoviridae is the only known virus family about which we have such profound ignorance. We do not even understand the maintenance strategies employed in nature by the agents, and we know much less about the resulting diseases, their pathogenesis, and detailed virology. The information gathered during control efforts directed toward recent epidemics has provided considerable fundamental information about filoviruses.

A number of colleagues, both in the laboratory and in the field, agreed to prepare reports reflecting recent research, thus permitting this supplement to the *Journal of Infectious Diseases*, which provides a single source for substantial new, peer-reviewed information. We have somewhat arbitrarily divided the supplement into the categories of clinical observations; epidemiology and surveillance; ecology and natural history; virology and pathogenesis; experimental therapy; control, response, prevention; and conclusions. “Ebola,” however, is not just one Ebola: There are 4 distinguishable subtypes, whose phylogenetic tree is shown on page iii of this supplement [1–3]. Because the subtypes, which may even be different virus species, have differing properties, we have grouped the papers by the subtype discussed within each subject area.

Marburg, the First Known Filovirus

Biomedical science first encountered the virus family Filoviridae when Marburg virus appeared in 1967 [4]. At that time, commercial laboratory workers with a severe and unusual disease were admitted to a hospital in Marburg, Germany. The attending physician recognized the distinctive clinical picture as additional cases appeared, and an investigation led to the isolation and identification of the immediate source of the virus as green monkeys imported from Africa for use in research and vaccine production. The monkeys, some of which had been shipped to Frankfurt, Germany, and Belgrade, Yugoslavia, were euthanatized, and the epidemic was contained with only 31 human cases and one generation of secondary transmission to health care workers and family members. Nevertheless, the bizarre morphology of the virions, the 23% human mortality, and the failure to identify the natural history of the virus left fear among many who were concerned with the role of viruses in human economy. Quarantine procedures were put in place in many countries to prevent the recurrence of disease introduced by imported monkeys [5], and tests were instituted to exclude Marburg virus from vaccine substrates [6]. Fortunately, there have been only three detected recurrences of Marburg virus, all in humans traveling in rural Africa, and none of these has led to extensive transmission [7–9]. This brief history of Marburg virus presages the very similar course of events with Ebola virus.

Ebola, the Second Known Filovirus

Humans Meet Ebola Virus in Africa, 1976

In the late 1970s, the international community was again startled, this time by the discovery of Ebola virus [10] as the causative agent of major outbreaks of hemorrhagic fever in the Democratic Republic of the Congo (DRC) [11] and Sudan [12]. International scientific teams that arrived to deal with these highly virulent epidemics found that transmission had largely ceased; however, they could reconstruct considerable data from the survivors. Medical facilities had been closed because of the high death toll among the staff, thus eliminating major centers for dissemination of infection through the use of sterilized needles and syringes and the lack of barrier-nursing techniques. In contrast, patients in the affected villages were segregated through traditional methods of quarantine, a step that controlled the situation outside the clinics. Much of the information concerning these outbreaks has been previously summarized [13].

The international alarm and research efforts that arose in response to these outbreaks quickly dwindled when the only convincing evidence that Ebola virus infections were continuing among humans consisted of a small outbreak in the Sudan in 1979 [14] and 1 case in Tandala, DRC, in 1977 [15].

Ebola Virus Visits the United States: The Virus Family Grows

In 1989, Ebola surprised us once more when it appeared in monkeys imported into a Reston, Virginia, primate facility outside of Washington, DC. Epidemics in cynomolgus monkeys (*Macaca fascicularis*) occurred in this facility and others through 1992 [16–17] and recurred in 1996, as reported in this supplement [18]. Epidemiologic studies that were conducted in connection with both epidemics [19, 20] successfully traced the virus introductions to one Philippine exporter but failed to detect the actual source of the virus. Attempts to work in the remote areas where the monkeys were captured have been too dangerous due to political instability. We do know that this virus strain (EBO-R) has an apparent Asian origin and lesser pathogenicity than other Ebola subtypes for both macaques and

After Ebola hemorrhagic fever (EHF) appeared in Africa in 1976–1979, it was not seen again until 1994. Was it “gone” during those 15 years? In one sense, certainly not—it was circulating in its natural reservoir. Was the virus causing sporadic human infections that remained undetected because the patients never contaminated hospitals to produce the savage nosocomial epidemics that brought Ebola virus to medical attention? During 1981–1985, Ebola virus surveillance was carried out concurrently with intensified efforts to understand monkeypox [29]. This surveillance may have identified several cases and estimated the seroprevalence among the population; however, the findings are subject to caveats because of problems with the validity of laboratory tests [27]. Serosurveillance in 1995 also suggested that human infections may have occurred from time to time [30].

During 1994–1996, no less than five independent active sites of Ebola virus transmission were identified: Côte d’Ivoire in 1994 [31]; DRC in 1995 [32]; and Gabon in 1994, 1995, and 1996 [3, 33–35]. The previously known Zaire subtype of Ebola virus (EBO-Z) and the newly discovered Côte d’Ivoire subtype (EBO-CI) were both involved, and as in previous African Ebola virus transmissions, the sites were in or near tropical forests, such as along riverine forests. Whether this hiatus after 1976–1979, which was followed by renewed human transmission, reflects actual Ebola virus activity or rather publicity combined with fortuitous entry of the virus into medical facilities (leading to recognition) is unknown; we believe the renewed quiescence of reported Ebola activity since 1996 argues for the former.

EBO-CI was discovered when ethologists in the Taï forest of Côte d’Ivoire noted that members of a chimpanzee troupe began to experience an unusually high mortality. One of the study group scientists became infected and was transferred to Basel, Switzerland, for definitive care [31]. The clinical information derived from her hospitalization provides the best-studied clinical case of any Ebola virus infection [36]. Furthermore, the circulation of virus in the well-defined region of the Taï forest reserve provides an excellent opportunity to study the Ebola reservoir question. Reports of the clinical case [36], the epidemiology in chimpanzees [37], and the pathogenesis in chimpanzees [38] are in this supplement.

EBO-Z was also circulating in Gabon [34], and at least 3 separate outbreaks in humans and nonhuman primates occurred [3]. Thus, Gabon may well provide another site where the search for risk factors of human infection and the natural reservoir could be carried out. Notable among the epidemics were features such as the important role of a dead, naturally infected chimpanzee in bridging the virus to humans, the rapid control of human transmission when barrier-nursing measures were instituted and the continued circulation of virus without these precautions, and the deep forest exposures of index cases.

EBO-Z, Kikwit, DRC, 1995

The description of the large African EHF outbreaks in 1976 was largely based on retrospective information, so the Kikwit epidemic provided a better opportunity for more detailed investigations while the epidemic was in progress. Other differences were also present. For example, in 1995, the press and tabloid response in Kikwit was extraordinary and unanticipated. The last weeks of this epidemic took place in an unprecedented atmosphere of legitimate news reporting and tabloid exploitation. Largely because of the popular success of Richard Preston’s book, The Hot Zone [39], there was tremendous public interest in both the information and misinformation that was generated by the media. Fortunately, careful mainstream journalists were accurate in carrying the best scientific information, and the World Health Organization (WHO) became a highly capable center for the dissemination of reliable facts about the epidemic [40]. Large donations flowed into WHO and directly to the DRC; however, there were difficulties with the disbursement of relief supplies and resources, acquisition of appropriate types of materials, and triage of the contributions.

Clinical disease. The clinical syndrome seen among patients in Kikwit resembled that seen in 1976 [13, 11], but bleeding was less common and other significant findings were identified, as reported by Bwaka et al. [41]. Unfortunately, circumstances did not permit as close an evaluation as would have been desirable. It was possible, however, to make observations on eye complications [42], pregnancy [43], late sequelae [44], and an unusual case with mucormycosis complicating Ebola [45]. As the epidemic progressed, mortality progressively declined from virtually 100% to 69% [46].

There is no known therapy for EHF. Late in the epidemic, this fact motivated a clinical experience with blood transfusions from survivors [47]. Although no contemporaneous controls are available for comparison, only 1 of the 8 treated patients died. Comparison with the bulk of patients in the outbreak, taking into account the patient’s age and sex, the day of treatment, and the stage of the epidemic, did not suggest any real benefit to the therapy [46]. In addition, virologic analysis of the incomplete specimen set that was available did not lend support for efficacy. Nevertheless, it is useful to consider what was present in the transfused blood that might have been helpful. It is questionable whether antibodies would have had much effect (see below), but the activated allogeneic lymphocytes and the added volume of platelets, erythrocytes, and plasma
were probably beneficial. If therapeutic studies are undertaken in patients in the future, it will be important to have randomized control serial laboratory samples and some consideration given to the potential immunostimulatory effects of allogeneic lymphocytes.

We assume that filoviruses, like other viruses causing hemor-rhagic fevers, can latently or chronically infect their natural reservoir hosts [48]. Primates seem to be susceptible hosts, and nonhuman primates may even provide a frequent link to humans. They are unlikely, however, to be the true reservoir hosts, given the high pathogenicity of filoviruses for African monkeys, macaques, chimpanzees, and perhaps great apes [4, 13, 16, 21, 34, 37, 38, 49, 50]. Furthermore, a direct search for chronic, persistent, or latent infection in monkeys was unsuccessful [51].

Marburg virus has been cultured from secretions or immunologically privileged sites 1–3 months after acute disease [4]. In the 1995 Ebola outbreak in Kikwit, late transmission of disease was not detected in follow-up of contacts of several survivors [44]. There was, however, evidence for Ebola virus RNA shed in semen and vaginal secretions for months [44, 52], although it was not possible to isolate virus. The only testicle examined from a fatal case showed clear-cut viral invasion [53].

Patients with persistent arthritis also had higher anti-Ebola IgG ELISA titers, suggesting increased or prolonged antigenic stimulation. Thus, although the question is not settled, persistence of virus or viral antigen or genomes for weeks into convalescence seems common, but long-term infection is apparently not likely.

IgG and IgM ELISAs [27, 27a] were used to evaluate the possibility of subclinical infections among family contacts [54], contacts of convalescent patients [44], medical staff [55], and local residents [30], and evidence suggested that a very low level of subclinical transmission occurred during the outbreak. Of interest, there was an appreciable seroprevalence among the residents of Kikwit and those of surrounding villages, which was thought to represent temporally distant infections [30].

Epidemiology and surveillance. The presence of the international teams allied with several organizations from the DRC during the end of the epidemic provided an opportunity for several studies to better define the transmission of Ebola virus among humans. Details of transmission in households [54] showed the important role of close contact and exposure to body fluids, particularly to care givers, who suffered the major burden of secondary infections. Touching cadavers at funerals was also an independent risk factor for disease and may well be related to the extensive skin involvement of Ebola virus, as discovered by Zaki et al. [53].

There is considerable misunderstanding concerning the potential for aerosol transmission of filoviruses. The data on formal aerosol experiments leave no doubt that Ebola and Marburg viruses are stable and infectious in small-particle aerosols, and experience of transmission between experimental animals in the laboratory supports this [49, 56–63]. Indeed, during the 1989–1990 epizootic of the Reston subtype of Ebola, there was circumstantial evidence of airborne spread of the virus, and supporting observations included suggestive epidemiology in patterns of spread within rooms and between rooms in the quarantine facility, high concentrations of virus in nasal and oropharyngeal secretions, and ultrastructural visualization of abundant virus particles in alveoli [17, 50]. However, this is far from saying that Ebola viruses are transmitted in the clinical setting by small-particle aerosols generated from an index patient [64]. Indeed patients without any direct exposure to a known EHF case were carefully sought but uncommonly found [65]. The conclusion is that if this mode of spread occurred, it was very minor.

What then were the major routes of transmission? Nonhuman primate studies [66] found conjunctival and oral routes of infection to be possible. It seems likely that the increased risk from late-stage patients [54] reflects increased virus excretion as the disease progresses, similar to that seen in monkey models [50]. Thus, mucous-membrane exposure, pharyngeal contamination during swallowing, inoculation via small skin breaks, or even infection from swallowed infectious material may all contribute to virus transmission.

Ecology and natural history. The epidemic also provided an opportunity to search for the elusive reservoir of Ebola virus in connection with an acute outbreak. It is not widely appreciated that there is only one reported study of any search for the reservoir [7]. Many ecologic investigations have been made in connection with other viruses [67]; it is not known if the methods used would have detected Ebola virus, although EBO-Z is lethal in the most commonly used assay system, the sucking mouse [68]. Certainly, antibodies were not sought.

In Kikwit, investigators were faced with multiple dilemmas, particularly timing and selection strategy. The first problem was that the outbreak began in January during the rainy season, but because of the delay in recognition and other factors, no research effort was mounted until several months later during the dry season [69]. At that time, there was no guarantee that the virus would still be present in its natural habitat. Several international teams began the search as soon as possible and made a broad general collection [70, 71]. The implicated index case [69] was a charcoal maker who lived in the city of Kikwit. He rode his bicycle through the savanna to an area of secondary forest, where he had exposure to tree-top, ground-level, and burrowing species of plants and animals. To complicate matters, he also had a small agricultural plot near a primary forest in a clearing near a stream. A decision was made to throw a wide net and capture arthropods and vertebrates from several biotopes, recognizing that the diversity of tropical species would be a limiting factor. Unfortunately, no evidence of Ebola or antibodies reactive with the virus were found in vertebrates, and Ebola genomes were not amplified from the extensive arthropod collections [70, 71]. Another group (R. Swanepoel and colleagues) from the National Institute of Virology in San-
dringhan, South Africa, returned to the area during the following rainy season and made selective collections to extend the available information (unpublished). The interpretation of these studies was augmented by the results of work done during 1979–1980 in connection with the monkeypox surveillance program [72].

A second problem was the choice of how to select the species sampled. There are several hypotheses concerning the most likely reservoirs for filoviruses [73], but each of these involves implicit assumptions about the nature of the reservoir [74]. It was known in 1995 that arthropod cells and arthropods themselves were not readily infected with filoviruses [75], and these observations have been extended [76, 77]; however, one must ask if the correct arthropod has been tested.

Virology and pathogenesis. Ksiazek et al. [27] provide the first opportunity to examine more than anecdotally the clinical virology of Ebola virus. The most important finding was that acutely ill patients are intensely viremic and that ELISA determination of viral antigens in serum provides a sensitive and specific way to quickly screen large numbers of suspect human samples. Virus isolation and reverse transcriptase–polymerase chain reaction are useful in a few instances as well [1, 28]. These results closely parallel those found in Ebola (Reston subtype) virus studies in naturally infected monkeys [50]. Antibodies appear as patients recover; this provides an illuminating contrast to hantavirus diseases, in which the onset of the detectable immune response coincides with the development of serious disease [78, 79].

There is no more contentious issue in Ebola virology than the measurement of simple IgG antibodies to assess past infection. Since 1976, indirect fluorescent antibody tests have been used for acute diagnosis and seroepidemiology, but their limitations were recognized early on [80]. During the Reston epidemics, the situation became more difficult. Monkeys with no likelihood of Ebola infection had positive titers, the titers could rise from negative to high levels, such as 1:256 in an animal under observation, and application of Western blots failed to resolve the problem [81]. The virus is not readily neutralized by convalescent sera, and no hemagglutinin has been detected, eliminating two of the common confirmatory tests. An ELISA test [27] appears to eliminate the false-positive results widely seen in normal monkeys and shows positive reactions with every monkey serum from a confirmed infection and with sera obtained from a small number of human Ebola survivors available at the time of test development. This outbreak provided an opportunity to apply the test to humans with acute infection [27]. The use of the test in such sera was satisfactory and provides an improved measure of Ebola antibodies, but more experience is indicated.

During acute disease, there was mRNA evidence of activation of multiple cytokines [82]. These cytokines have been implicated in the pathogenesis of several forms of shock and cause specific defects in vascular permeability in filovirus infections studied in vitro [83]. Another interesting finding in acute-phase infections of humans and nonhuman primates [28] was the presence of a circulating soluble glycoprotein, which shares ~300 amino acids with the viral glycoprotein that is produced through transcriptional editing of the same gene [1]. It has been speculated that this protein may serve as some form of immunologic decoy, preventing an effective immune response. There are several other possible immunosuppressive mechanisms (reviewed in [2]), including the extensive necrosis of spleen and lymph nodes from fatal human and nonhuman primate cases.

Extensive infection and co-localized necrosis were found in parenchymal cells, macrophages, and endothelial cells of many organs [53, 84]. The virologic and pathologic findings are important for the way we think about therapy of patients infected with EBO-Z. The pathogenetic hurdle is the extensive nature of infection with a cytopathic virus and the lack of an effective immune response. In fact, the infection and related necrotic lesions are so widespread in fatal cases that it seems unlikely that supportive care will have much impact on survival unless some form of antiviral or immunologic therapy can be instituted relatively early in disease. The extensive cytokine activation explains some features of the disease, and it may well be that disseminated intravascular coagulation occurs on the severely affected endothelial cell surfaces, as seen in some animal models, but these are not the driving forces behind the fatal disease process. Treatment of these phenomena as well as traditional supportive care may be useful in some cases but should not distract research energies from antiviral drugs, effective passive antibody, or other forms of therapy designed to modify the underlying problem.

Experimental therapy. Fortunately, there are examples of provocative new findings that may provide therapy for filovirus infections. Simple convalescent serum has generally had low neutralizing capacity in vitro and has not conferred protection on passive transfer. Nevertheless, high-titered hyperimmune horse anti-Ebola serum has been produced and been found protective in baboons challenged with Ebola virus [85]. This product has been confirmed to be efficacious in guinea pigs, but it is not as useful in rhesus monkeys or the mouse model of Ebola virus infection [86, 87]. The production of human monoclonal antibodies against Ebola virus surface protein from mRNA extracted from bone marrow of Kikwit survivors raises the possibility that an improved, standardized, safe, and replenishable source of therapeutic antibodies could be developed [88].

Although there is no obvious role for an Ebola virus vaccine today, there are promising efforts toward experimental filovirus vaccines [89, 90]. It is important to continue these studies to be sure we have the technology to produce a vaccine should it be needed and to elucidate mechanisms of protection to help us in the search for effective immunotherapeutic agents.

Antiviral drugs also show promise in experimental infections [91]. The thrust of the effort is a collaborative approach among the US Army Medical Research Institute of Infectious Diseases
(Fort Detrick, Frederick, MD), the National Institute of Allergy and Infectious Diseases (National Institutes of Health, Bethesda, MD), and other partners to identify drugs efficacious against viruses such as respiratory syncytial virus (RSV), another negative-sense, single-stranded RNA virus with some similarities to Ebola. The expense of preclinical and early clinical work would be justified by the potential commercial use of the drug against RSV, while the efficacy against Ebola virus would provide an alternate model to demonstrate broad-spectrum preclinical efficacy.

Control, response, and prevention. The epidemic in Kikwit posed certain serious problems. The medical infrastructure was poor to begin with and suffered greatly from the epidemic. Hospitals were closed, and 30% of the physicians and 10% of the nurses contracted EHF. The city, with a population of >250,000, had no regular transportation, a paucity of vehicles, no newspaper or radio station, and no reliable electric power. Because of fear and of stigmatization, new cases were cared for at home and often in secrecy. It became urgent to rehabilitate the medical infrastructure and to convince patients to come to the hospital, where they could be isolated and their families could be observed. This contrasts with the 1976 outbreaks, which occurred in villages where leaders enforced quarantine in local houses. This action, combined with the collapse of the medical care system, effectively ended the epidemics. The importance of the medical care facility in amplifying the spread of Ebola virus is emphasized by the fact that only the hospitals in Kikwit and Mosango, DRC, had extensive transmission: ~7% of patients left for small villages, and no transmission was noted there [69]. We do not know if Ebola virus transmission would have continued indefinitely, burrowed into the mass of people in the city, if measures to begin hospital use had not succeeded. In any case, the infection of health care workers [69, 55] ended with the arrival of proper patient-isolation supplies and training in barrier-nursing techniques [55, 69, 92]. The coordination of medical logistics and plans for rational triage of the patients were key in the effort [93, 94].

The lesson is obvious: The hospital is the link that must be strengthened. This will require both money and training, but the improvements will be useful in preventing many other infections, including those with hepatitis C and human immunodeficiency viruses. How this might occur without marked economic and cultural changes is not clear; despite intensive training, health care workers in Kikwit abandoned most of the improvements in medical hygiene within 3 months of the end of the epidemic, due in part to a lack of supplies and a reversion to previous practices. Unfortunately, the massive aid that comes with emergencies does not continue in reduced form to help prevent future emergencies.

Surveillance is also a problem. The case definition that was adopted was accurate in the epidemic setting, but it would be much less so in sporadic infections or at the beginning of an epidemic [69]. The finding of copious amounts of Ebola virus antigen in skin opened the way to confirm cases by taking simple skin biopsies, which could be placed in formalin and analyzed later by immunohistochemistry [53, 92]. This obviates the need for cold chain or special precautions while processing or shipping infectious material. One could argue that Ebola diagnostics should be placed at many sites in the potentially endemic areas, but this may be unrealistic given the small number of expected cases and the economics. Such tests could be devised on the basis of antigen detection on paper strips [27, 27a], but the logistics of production, distribution, quality control, and shelf-life considerations are formidable. It would seem that laboratory capability for diagnosing common diseases, such as shigellosis or typhoid, would be much more practical as a first step. This would also make it easier to sort out which patients to suspect for Ebola virus infection, remembering that the initial diagnosis of the epidemic in Kikwit was bloody diarrhea until clinicians suspected otherwise weeks later [95].

Thus, the algorithm proposed by Lloyd et al. [92] calls for early recognition of suspect cases by the clinician and then institution of simple, inexpensive barrier-nursing precautions. If a patient dies, as the majority of humans infected with EBO-Z do, a skin biopsy is obtained and sent for analysis. Today, the Centers for Disease Control and Prevention is the main site for analysis, but as demand builds, the technology can be transferred to regional centers. The delay in definitive diagnosis is not the problem one might anticipate. The protocol calls for barrier nursing to begin at once, thus decreasing the chance of spread. In the Kikwit outbreak, almost 5 months passed from the beginning of the outbreak until the first samples were obtained. The ability to obtain a skin sample safely and without cold chain problems should encourage more use of this technique and may well give an earlier warning.

Information for the Future

What information is needed to deal with Ebola virus? The major questions are tied to important issues in biology. For example, how will we be able to elucidate the natural reservoir without intensive studies of the many animals resident in the tropical forest? Do we really have a viable hypothesis as to the true reservoir? Certainly we would put bats high on our list of suspects [77], but should we do this to the exclusion of other species [74]?

Very little is known about the virology of Ebola. For example, this agent has less than a dozen genes, compared with the expansive genomes of poxviruses or herpesviruses. How does it accomplish its task of natural maintenance and also cause disease in humans? The virus appears to be relatively refractory to the antiviral effects of interferon [75], but the mechanism is unknown. Recent studies have shown that the virus inhibits gene induction by interferons and double-stranded RNA in endothelial cells, effects that could be relevant to pathogenesis [96].
We have no structural studies of the virus that could yield information on the function of some of the unmapped genes. Additional knowledge of the three-dimensional structure of the virus and its proteins could be helpful in designing inhibitors of the fusion process [97] and of the polymerase. The clinical description of the disease is still incomplete, due in part to the lack of infrastructure during epidemics and the difficult and dangerous circumstances. For example, does pancreatitis contribute to the demise of patients [36]? What are the variations in clinical pathology findings? Much additional work on the virology and immunology remains.

Finally, we need a pre-planned response team that is already integrated, prepared to execute selected functions, and equipped. This team may have to wait 20 years for the next epidemic; however, its chance to respond may come much sooner.

Information for Now

Most of the known information on filoviruses can be found in Marburg Virus Disease [4], Ebola Virus Haemorrhagic Fever [13], and this supplement. Marburg and Ebola Viruses [98] has just been published and contains, but is not limited to, particularly good summaries of recent work on the molecular biology of filoviruses. Much of the Russian literature has never been properly surveyed and synthesized in the English language. This supplement contains reviews of Russian work on antibody therapy [85] and pathogenesis [99], and other information on pathogenesis [100] and vaccine immunology is available [101].

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References


