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Treatment of Marburg and Ebola hemorrhagic fevers: A strategy for testing new drugs and vaccines under outbreak conditions

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Abstract

The filoviruses, Marburg and Ebola, have the dubious distinction of being associated with some of the highest case-fatality rates of any known infectious disease—approaching 90% in many outbreaks. In recent years, laboratory research on the filoviruses has produced treatments and vaccines that are effective in laboratory animals and that could potentially drastically reduce case-fatality rates and curtail outbreaks in humans. However, there are significant challenges in clinical testing of these products and eventual delivery to populations in need. Most cases of filovirus infection are recognized only in the setting of large outbreaks, often in the most remote and resource-poor areas of sub-Saharan Africa, with little infrastructure and few personnel experienced in clinical research. Significant political, legal, and socio-cultural barriers also exist. Here, we review the present research priorities and environment for field study of the filovirus hemorrhagic fevers and outline a strategy for future prospective clinical research on treatment and vaccine prevention.

Keywords: Ebola virus; Ebolavirus; Marburg virus; Marburgvirus; Filovirus; Viral hemorrhagic fever; Clinical research; Treatment; Vaccine; Emerging infectious disease; Biodefense

1. Introduction

The filoviruses are nonsegmented, single-stranded negative-sense RNA viruses with an unusual filamentous morphology. The family *Filoviridae* is divided into two genera, *Ebolavirus* and *Marburgvirus*. Four species of ebolavirus (Zaire, Sudan, Ivory Coast and Reston) and one of marburgvirus (Lake Victoria marburgvirus) are recognized (Table 1). Filoviruses circulate in sub-Saharan Africa where they occasionally cause large outbreaks of severe hemorrhagic fever (Fig. 1) (Bausch, 2007a). The natural reservoir of the filoviruses remains unknown, although bats are suspected (Leroy et al., 2005; Swanepoel et al., 2007; Towner et al., 2007). Transmission between humans results from direct contact with infected blood and bodily fluids (Dowell et al., 1999; Bausch et al., 2007b). No specific antiviral therapies or vaccine are yet available. The primary control strategy relies on thorough case identification and contact tracing, with immediate isolation of suspected cases in specialized isolation wards (CDC/WHO, 1998; Bausch, 2007b).

Increasing frequency of natural transmission of the filoviruses in sub-Saharan Africa, as well as concerns about bioterrorism and imported cases, have heightened their importance to public health over the past few decades. These concerns have fostered intense laboratory-based research efforts in industrialized countries on the pathogenesis, treatment, and vaccine prevention for filovirus hemorrhagic fever (FHF) that hold the potential to reduce case-fatality rates and drastically curtail outbreaks (Schuler, 2005). Several candidate therapies have shown efficacy in nonhuman primates if initiated soon after virus challenge, and a number of vaccines have been developed that protect these animals against otherwise uniformly lethal infection.

The research advances on treatments and vaccines for FHF may soon render products ready for clinical testing. However, while the basic science stages of research takes place largely in the controlled environment of high-containment laboratories, if clinical research on FHF is to be carried out it must occur in

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Table 1
Laboratory-confirmed cases and outbreaks of filoviral hemorrhagic fever

Virus and date of onset of transmission	Epicenter(s)	Source of primary infection	Factors contributing to spread	#Cases	CFR (%)
Marburgvirus					
1967	Marburg and Frankfurt, Germany; Belgrade, Yugoslavia (present Serbia)	Imported monkeys from Uganda	Dissection of monkeys to harvest organs, nosocomial transmission	32	22
1975	Rhodesia (present Zimbabwe)/South Africa[a]	Unknown	Nosocomial transmission	3	33
1980	Kisumu and Nairobi, Kenya	Exposure in cave? Monkey contact?	Nosocomial transmission	2	50
1987	Mombasa, Kenya	Exposure in cave?	–	1	100
1998	Durba, DRC	Exposure in gold mine	Repeated primary introductions into humans	154	83
2004	Uíge, Angola	Unknown	Nosocomial and community-based transmission	252	90
2007	Kamwenge, Uganda	Exposure in gold mine?	Presumed primary introductions in 2 cases, with subsequent person–person spread	4	25
Zaire ebolavirus					
1976	Yambuku, Zaire (present DRC)	Unknown	Nosocomial transmission	318	88
1977	Tandala, Zaire	Unknown	–	1	100
1994	Ogooué-Ivindo Province, Gabon	Unknown	Traditional healing practices, nosocomial and community-based transmission	49	59
1995	Kikwit, DRC	Unknown	Nosocomial transmission	315	81
1996	Ogooué-Ivindo Province, Gabon	Consumption of dead chimp	Secondary spread to caregivers	31	68
1996	Ogooué-Ivindo Province, Gabon	Unknown	Exposure while hunting, traditional healing practices	60	75
1996	Johannesburg, South Africa	Imported from Gabon by infected doctor	Nosocomial transmission	2	50
2001	Ogooué-Ivindo Province, Gabon and Cuvette Ouest Region, ROC	Hunting and consumption of nonhuman primates	Exposure while hunting, secondary spread to caregivers, traditional healing practices	124	78
2002	Cuvette Ouest Region, ROC	Hunting and consumption of nonhuman primates	Exposure while hunting, secondary spread to caregivers	143	89
2003	Cuvette Ouest Region, ROC	Hunting and consumption of nonhuman primates	Exposure while hunting, secondary spread to caregivers	35	83
2007	Kasai Occidental Province, DRC	Unknown	Unknown	264[b]	71
Sudan ebolavirus					
1976	Maridi and Nzara, Sudan	Unknown	Nosocomial transmission	284	53
1979	Nzara, Sudan	Unknown	Nosocomial transmission	34	65
2000	Gulu, Uganda	Unknown	Nosocomial and community transmission	425	53
2004	Yambio, Sudan	Unknown	Unknown	17	41
Ivory Coast ebolavirus					
1994	Taï Forest, Côte d'Ivoire	Necropsy of chimpanzee	–	1	0
Ebolavirus, species undetermined[c]					
2007[c]	Bundibugyo District, Uganda	Unknown	Unknown	149[c]	25

Laboratory-acquired infections and infections due to Reston ebolavirus, which has not been epidemiologically linked to Africa or associated with human disease, are excluded. Abbreviations: CFR: case-fatality rate, DRC: Democratic Republic of the Congo, ROC: Republic of the Congo.

[a] The outbreak was initiated in travelers who passed through Zimbabwe on their way to South Africa. The place where infection occurred is unknown.

[b] Although a total of 264 cases and 187 deaths were reported by the DRC Ministry of Health, simultaneous outbreaks of malaria, typhoid, and shigellosis make it difficult to ascertain the percentage of these actually attributable to Ebola hemorrhagic fever. Only 26 cases were laboratory confirmed.

[c] This outbreak is still ongoing at the time of writing of this article. Initial reports attribute it to a new species of ebolavirus, but details are not yet available. Case numbers and the case-fatality rate are up until January 4, 2008.

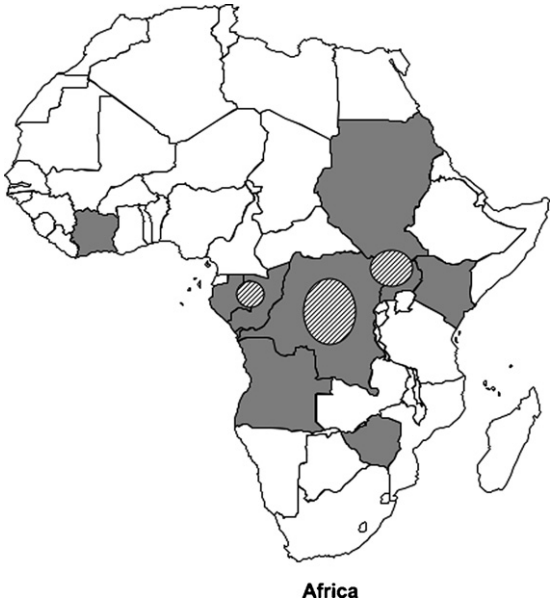

Fig. 1. The known distribution of human cases of filoviral hemorrhagic fever (FHF). Countries where human cases of Marburg or Ebola hemorrhagic fever have been confirmed are noted in gray. Areas where more than one outbreak have been noted are indicated by cross-hatching, and include the border area of Gabon and the Republic of the Congo, central Democratic Republic of the Congo (DRC), and northern Uganda/southern Sudan/northeastern DRC.

endemic areas in sub-Saharan Africa, most likely under outbreak conditions in areas with rudimentary medical infrastructures. In fact, any plan to conduct prospective clinical research on FHF must deal with a staggering array of scientific, logistical, political, social, financial, legal, and ethical challenges. Here, we review the progress made to date in understanding the pathogenesis, clinical presentation, treatment, and vaccine prevention of FHF, then describe the settings in sub-Saharan Africa where field research on FHF must take place, and finally outline a strategy for prospective clinical research on treatment and vaccine prevention in this challenging environment.

2. Research progress

A decade of work by teams at the Centers for Disease Control and Prevention (CDC), the US Army Medical Research Institute for Infectious Diseases, the Public Health Agency of Canada and other laboratories has led to major progress in understanding the pathogenesis of the FHFs and developing experimental therapies and vaccines. Studies have been based on models of infection in mice, guinea pigs, and nonhuman primates, supplemented by a limited amount of clinical data obtained from humans during outbreaks in Africa.

2.1. Pathogenesis

The major clinical features of FHF appear to result from an intense systemic inflammatory response resembling septic shock (Bray and Mahanty, 2003; Mahanty and Bray, 2004). Studies in nonhuman primate models have shown that monocytes, macrophages and dendritic cells, the ubiquitous "sentinels" that normally guard the body against microbial invasion, are the major sites of initial viral replication and play the central role in pathogenesis (Schnittler et al., 1993; Zaki and Goldsmith, 1999; Bosio et al., 2003, 2004; Geisbert et al., 2003b; Bray and Geisbert, 2005). Virus is then distributed by circulating monocytes/macrophages to a wide variety of organs and cell types. The system-wide release of proinflammatory cytokines and chemokines by these infected cells causes fever, vascular instability, hypotension and shock, and ultimately multi-organ system failure (Mahanty and Bray, 2004; Hoenen et al., 2006). The synthesis of cell surface tissue factor triggers the extrinsic coagulation pathway (Hensley et al., 2002; Geisbert et al., 2003c; Hensley and Geisbert, 2005). In nonhuman primate models of EHF, disseminated intravascular coagulation is noted by the second day post-infection, as evidenced by the appearance of D-dimers in the plasma (Geisbert et al., 2003c). Extensive necrosis is noted, especially in the liver, although it is usually not to a degree to account for death (Egbring et al., 1971; Martini, 1971; Stille and Bohle, 1971; Gear et al., 1975; Geisbert and Jaax, 1998; Zaki and Goldsmith, 1999). Although endothelial cell infection is consistently noted on post-mortem tissues collected from filovirus victims (Zaki and Goldsmith, 1999), studies in nonhuman primates suggest that this does not occur until late in the course of the disease (Geisbert et al., 2003d).

The suppression of host-immune responses is a key component of filovirus pathogenesis, especially for Ebola virus infection, where two virus proteins have been shown to suppress interferon responses (Basler et al., 2003; Reid et al., 2006). Furthermore, in most fatal cases, patients fail to produce antibodies against the filovirus and die with persistent high viremia (Ksiazek et al., 1999; Sanchez et al., 2004). Because the initiation of an adaptive immune response requires that dendritic cells present viral antigens to lymphocytes, the explanation is readily available; dendritic cells are a major site of filoviral replication, which blocks their maturation and causes their death through necrosis (Geisbert et al., 2003b; Mahanty et al., 2003). Although lymphocytes remain free of infection, they are destroyed in massive numbers over the course of illness through apoptosis, again similar to the process seen in septic shock (Baize et al., 2000; Geisbert et al., 2000; Hotchkiss and Karl, 2003). Thus, anti-apoptotic therapies that can preserve immune function might prove to be future therapies for FHFs (Parrino et al., 2007).

2.2. Clinical presentation

Although increasingly organized surveillance and response systems for filovirus outbreaks in the last decade have allowed for more frequent clinical observation, there is still a dearth of detailed and systematically collected clinical data. With the exception of Reston ebolavirus, which does not appear to be pathogenic to humans, all the filoviruses appear to produce a similar illness. The presenting symptoms are difficult to distinguish from a host of other febrile illnesses. After an incubation period of around 8 days (range 3–21 days), disease typically begins with the abrupt onset of fever, chills, headache, general malaise, anorexia, sore throat, and chest, back, muscle, and joint pains (Martini et al., 1968; Egbring et al., 1971; Martini, 1971; Stille

and Bohle, 1971; Todorovitch et al., 1971; Gear et al., 1975; Pattyn et al., 1977; Anon., 1978; Smith et al., 1982; Nikiforov et al., 1994; Johnson et al., 1996; Bwaka et al., 1999; Bausch et al., 2006; Colebunders et al., 2007). Conjunctival injection or hemorrhage is seen in up to half of patients at presentation, but is not typically accompanied by itching, discharge, or rhinitis. Patients themselves rarely refer to problems with their eyes, other than the cosmetic complaint of redness. A fleeting maculopapular or morbilliform rash is sometimes noted over the thorax, face and arms, especially in Caucasians. A dry cough, sometimes accompanied by a few scattered rales on auscultation, is frequently noted, but prominent pulmonary symptoms or the presence of productive sputum early in the course of disease are uncommon. Jaundice is not typical and should suggest a different diagnosis.

Gastrointestinal signs and symptoms develop within the first few days of illness, including nausea and vomiting, epigastric and abdominal pain and tenderness (especially in the right upper quadrant in Ebola hemorrhagic fever (EHF)), and diarrhea. FHF has sometimes been mistaken for acute appendicitis or other abdominal emergencies. Hepatosplenomegaly is frequently seen, but it is unknown whether this is specific to FHF or simply represents the high underlying prevalence of hepatosplenomegaly in populations in sub-Saharan Africa where most clinical observations have been made.

In severe cases, vascular instability develops, usually 4–5 days after the onset of symptoms, and may be evidenced by facial flushing, edema, proteinuria, bleeding, hypotension, and shock. Hemorrhage is most often gastrointestinal in nature (vomiting blood or blood passed from the rectum), but vaginal bleeding, petechiae, purpura, nose bleeds, and bleeding from the gums and venupuncture sites may be seen. Coughing up blood (hemoptysis) and hematuria are less frequent. Hemorrhage is almost never present in the first 2 days of illness. Central nervous system manifestations, including disorientation, gait anomalies, convulsions, and hiccups may be noted in end-stage disease.

Common clinical laboratory findings include WBC abnormalities (moderate leucopenia and lymphopenia with atypical lymphocytes early in the course of disease, with late leukocytosis and left shift), hemoconcentration, mild-to-moderate thrombocytopenia, blood coagulation abnormalities consistent with disseminated intravascular coagulopathy, and elevated hepatic transaminases (typically AST > ALT), amylase, blood urea nitrogen, and creatinine (Egbring et al., 1971; Martini, 1971; Stille and Bohle, 1971; Gear et al., 1975; Rollin et al., 2007). Radiographic examination has not been reported.

The clinical course of FHF usually unfolds quite rapidly, with death in fatal cases 7–10 days after symptom onset. Ebola Zaire and Marburg virus infection in sub-Saharan Africa are consistently associated with case fatalities of 80–90%, and Sudan ebolavirus 50–60% (Peters and Zaki, 2006). Mild and even asymptomatic cases have been reported (Leroy et al., 2001; Borchert et al., 2002). Common indicators of a poor prognosis include shock, bleeding, neurological manifestations, high viremia, and elevated levels of AST (Sanchez et al., 2004; Rollin et al., 2007). High maternal and fetal mortality are also noted in pregnant patients with FHF, in whom vaginal bleeding and spontaneous abortion usually occurs (Mupapa et al., 1999b).

2.3. Drug therapy

No licensed antiviral drug is efficacious against the filoviruses. Convalescent serum and whole blood have been used in a few instances, but their efficacy has never been convincingly demonstrated (Emond et al., 1977; Mupapa et al., 1999a). Laboratory studies of poly- and monoclonal antibodies have met with mixed results, although results from perhaps the most relevant animal model, cynomolgus monkeys, have not been encouraging (Jahrling et al., 1996, 1999, 2007; Wilson et al., 2000; Gupta et al., 2001; Takada et al., 2006; Oswald et al., 2007). Interferon-α, recombinant interferon, and extracorporeal blood treatment with hemosorbents and dialysis have been tried in a few patients who survived, but it is not clear that these measures were responsible for the favorable outcome (Emond et al., 1977; Nikiforov et al., 1994; Kudoyarova-Zubavichene et al., 1999). The nucleoside analog ribavirin, which has proven beneficial in some other viral hemorrhagic fevers (VHFs), has no effect against the filoviruses (Huggins, 1989).

Experimental therapies for the FHFs can be divided into those designed to directly block filovirus replication and those that act indirectly through the modification of deleterious host responses. In the first category are antisense oligonucleotides and short interfering RNA (siRNA) molecules that have become a focus of intense interest as therapies for a wide range of diseases (Spurgers et al., 2008; Warfield et al., 2006a). Antisense phosphorodiamidate morpholino oligomers (PMOs) targeting predicted sequences in the mRNA of 3 viral genes protected 75% of rhesus macaques against Zaire virus when treatment was begun before viral challenge (Enterlein et al., 2006; Warfield et al., 2006b). Treatment with siRNA has been successful in guinea pigs, but results have not yet been reported for nonhuman primates (Geisbert et al., 2006). Both PMOs and siRNA have so far proven safe in animal and human testing. Improved delivery systems and combinations of molecules targeting multiple viral genes may further improve the efficacy of these approaches. Certain other small molecules and licensed preparations of type I interferon are active *in vitro* and in rodent models, but not in nonhuman primates (Bray and Paragas, 2002). *S*-adenosylhomocysteine hydrolase inhibitors have also shown some success in the mouse model (Bray et al., 2000).

Treatments aimed at modifying deleterious host responses have derived from improved understanding of the pathogenesis of septic shock and its similarity to FHF. Recognizing that disseminated intravascular coagulation is triggered by the binding of tissue factor on the surface of infected macrophages to circulating factor VII, Geisbert et al. (2003a) attempted to block this interaction by giving daily injections of recombinant nematode anticoagulant protein C2 (rNAPC2), a product that is in advanced human trials for a number of applications. A 33% reduction in mortality was noted when 9 macaques were treated within 1 day after Ebola Zaire virus challenge. Most animals showed a marked reduction in coagulopathy and a striking decrease in levels of proinflammatory mediators. A 100-fold

drop in peak viremia was noted in the 3 surviving animals. The same therapy produced a lesser degree of benefit in macaques infected with the highly virulent Angola strain of marburgvirus (Geisbert et al., 2007).

Both septic shock and FHF are characterized by a significant fall in the plasma protein C level over the course of illness. Intravenous infusion of activated protein C has shown benefit for the treatment of gram-negative sepsis in large clinical trials (Fourrier, 2004). Administration of activated protein C to a group of 11 Zaire virus-infected monkeys resulted in a significant prolongation of the mean time to death and survival of 2 of the animals (Hensley et al., 2007).

Despite some promising results, it must be recognized that successful interventions have been initiated either before or soon after virus challenge. No therapy, licensed or experimental, has yet been shown to protect nonhuman primates against an otherwise lethal filovirus infection once disease has begun. The critical stages of disease progression and points at which intervention may be successful remain largely unknown.

2.4. Vaccines

A number of new vaccine candidates have been shown to protect nonhuman primates against otherwise uniformly lethal challenge with the filoviruses. The first successful approach used a prime-boost strategy in which a series of 3 injections of DNA encoding the nucleoprotein and surface glycoprotein of Zaire virus was followed by a single inoculation of a replication-defective recombinant adenovirus encoding the same antigens (Sullivan et al., 2000). Subsequent testing showed that the adenovirus-vectored vaccine was protective when given alone as little as 4 weeks before challenge (Sullivan et al., 2003). The DNA component has been shown to be safe and immunogenic in humans and the adenovirus product is also currently undergoing Phase I testing (Martin et al., 2006). Both the full prime-boost series and fast-acting adenovirus vaccine could play important roles in dealing with EHF; the former could be used to generate long-lasting immunity in laboratory workers and members of international outbreak response teams and the latter to protect local health workers at outbreak sites.

Another promising advance has been the development of live, recombinant vesicular stomatitis virus (rVSV) vaccines expressing the surface glycoprotein of ebolavirus or marburgvirus (Jones et al., 2005). A single injection of either product protected nonhuman primates against subsequent challenge with the corresponding pathogen, but no cross-protection was observed. These vaccines were also effective when given by the nasal or oral route, potentially facilitating their use in epidemics (Jones et al., 2006). Furthermore, the rVSV-Marburg vaccine prevented illness even when given 30 min after inoculation of a lethal dose of marburgvirus, suggesting that it may be useful as post-exposure prophylaxis (Daddario-DiCaprio et al., 2006). The rVSV-Ebola vaccine was also beneficial when given 30 min post-exposure, preventing the death of 50% of the animals (Feldmann et al., 2007). Such fast-acting vaccines could potentially be used as post-exposure prophylaxis after laboratory accidents or in the field when a clear exposure occurs. Although there are concerns over the safety of replication-competent rVSV-vectored vaccines in sub-Saharan Africa, where considerable numbers of immunocompromised persons with HIV/AIDS or malnutrition would likely be vaccinated, preliminary studies in immunocompromised nonhuman primates have not indicated problems (Thomas Geisbert, unpublished data).

A few other vaccine strategies for the FHFs are being explored; a live recombinant parainfluenza virus type 3 vaccine expressing the Ebola Zaire virus surface glycoprotein was shown to be protective when given by the intranasal route (Bukreyev et al., 2007). A preparation of noninfectious ebolavirus-like particles also protected laboratory primates, though a series of doses was required (Warfield et al., 2007).

3. Filovirus outbreaks—controlled chaos

Except for outbreaks related to the inadvertent importation of infected monkeys to Europe, cases of FHF in humans have been exclusively noted in sub-Saharan Africa (Table 1 and Fig. 1). Although seroprevalence data suggest that some degree of endemic transmission probably exists beneath the threshold of detection, especially for ebolaviruses (Monath, 1999; Bausch et al., 2003), filovirus transmission is generally recognized only in outbreak form (Bausch, 2007a). The largest outbreaks (300–400 cases) are invariably a result of nosocomial amplification. Thus, it is no surprise that they are typically seen in the most remote and resource-poor areas of sub-Saharan Africa where the healthcare delivery system and infection control standards have deteriorated. In most cases, the roots of this deterioration can be traced to years of civil unrest and violence. The patient-care setting often consists of multi-bed units, frequently without running water, a limited array of basic antimalarials, antibiotics, and analgesics, and an at-best rudimentary diagnostics and clinical laboratory. In practical terms, this translates to a dearth of personnel and supplies, with a typical scenario being an understaffed health center with undertrained workers without ready access to gloves and other personal protective equipment, sometimes tempted by the difficult circumstances to reuse contaminated needles and syringes. The physical infrastructure is often equally inadequate, with no stable electricity, refrigerators or freezers. Telecommunications and Internet access may be difficult or even non-existent, and transport to the distant major population centers limited and arduous.

Outbreaks begin when a human is infected through contact with the still unidentified primary reservoir (perhaps a bat) (Leroy et al., 2005; Swanepoel et al., 2007; Towner et al., 2007), or an infected nonhuman primate, with amplification resulting from secondary transmission between humans, which typically takes place when family members or healthcare workers are exposed to the patient's blood and bodily fluids. As the number of cases begins to mount, the community is confronted with a mysterious and fearful new scourge (Bausch, 2001). Traditional belief systems common in Central Africa often result in the disease being attributed to sorcery or poisoning rather than to a virus (Hewlett and Amola, 2003; Hewlett et al., 2005). Deaths of healthcare workers often occur (and are often a decisive clue that a filovirus is circulating), sometimes prompting other workers to

flee their posts, further destabilizing the already-fragile health-care delivery system (Bausch, 2001). Laboratory confirmation, which requires sending specimens to one of the few laboratories in the world with the necessary reagents and biosafety measures to perform filovirus diagnostics, usually lags. Consequently, a concerted international outbreak response is typically not mounted until months after the occurrence of the first case, which is almost always identified retrospectively (Bausch and Rollin, 2004; Bausch, 2007a). Finally, an outbreak control team composed largely of foreigners and government representatives from a distant capital descends upon the village. A flurry of activity ensues, with creation of committees and control programs. If clinical research on the filoviruses is to take place, it must occur in this tense and chaotic environment and be executed simultaneously and without compromising the multifaceted components of outbreak control.

Standard control measures for the filoviruses call for hospitalization of patients in an isolation ward (CDC/WHO, 1998; Bausch, 2006) (Fig. 2). This is the setting where future clinical research would logically take place. Ironically, in addition to the

Fig. 2. Isolation wards for patients with filoviral hemorrhagic fever (FHF). Clinical research on FHF in sub-Saharan Africa would most likely take place in isolation wards established during the course of outbreaks. The infrastructure and equipment available at these sites may vary drastically. Although virtually all are "make-shift" in response to an outbreak, some wards are set up in pre-existing hospitals and may benefit from other services available at the site, while others are situated in local health clinics or rapidly constructed de novo in remote areas and offer little in the way of modern medical care. Some examples are shown. Top row: exterior and interior of the EHF isolation ward, Kampungu, Democratic Republic of the Congo (DRC), 2007 (Courtesy of Médecins Sans Frontières, Belgium). Middle row: EHF isolation ward, Gulu Regional Hospital, Gulu, Uganda, 2000. Left: workers remove the body of a victim from the isolation ward. Right: interior of the ward (Photos by Daniel G. Bausch). Bottom row: Marburg hemorrhagic fever, Durba, DRC, 1998. Left: children cluster outside an isolation unit in the local health center. Right: workers in protective garb prepare to enter the ward (Photos by Daniel G. Bausch).

challenges presented by the aforementioned limitations in physical infrastructure and personnel, recruitment of patients, even in large outbreaks, could be a problem (Bausch et al., 2007a). The inherent severity of filovirus infection, absence of specific antifilovirus therapies, limited availability of supportive measures, and, at times, the channeling of energies and resources into community control rather than patient-care have all contributed to the extremely high case-fatality rate. An unfortunate result of this situation is that the isolation ward is frequently considered as a place where one goes to die, not survive. Furthermore, the environment of the isolation ward is unfamiliar and even threatening, with treatment often being administered by mask-, gown-, and glove-clad foreigners and contact with family members extremely limited. Death brings the possibility of being buried in an unfamiliar setting without the traditional rites so important in African culture, while those who survive to return to their communities are often ostracized (Boumandouki et al., 2005). Adding on the vestiges of colonial era suspicion of foreigners and present-day frictions between ethnic groups, it is not surprising that resistance to admission to the isolation ward is high, and sometimes even violent (Larkin, 2002). In the worst-case scenario, and if not conducted openly and with cultural sensitivity, clinical research in this charged setting could run the risk of meeting with the perception that foreigners and others in power are experimenting on vulnerable populations. However, measures now routinely implemented in the outbreak response to filoviruses, including the inclusion of social mobilization teams and concerted efforts to provide thorough explanation of the goals and movements of the outbreak response efforts to the local population, are the right steps to alleviating this concern (Formenty et al., 2003).

4. A strategy for bringing research products to field testing

The recent success in developing therapies and vaccines that protect laboratory primates against the filoviruses suggests that similar approaches may be effective in humans. However, the typical setting of a filovirus outbreak in Central Africa, the only place where human efficacy trials might be possible, presents an extreme contrast to the highly controlled, resource-rich environment of high-containment laboratories in industrialized countries. Although human efficacy trials might be avoided altogether through use of the Food and Drug Administration's (FDA) so called "animal rule" (Roberts et al., 2008), which allows for licensure of new drugs and biological products based on evidence of effectiveness derived from studies in well-characterized animal models when human efficacy studies are not ethical or feasible, it is likely that some evidence of efficacy in humans will still be needed before widespread use of any developed product. As discussed above, communities in Africa most affected by filoviruses and where treatments and vaccines are most needed, often harbor significant suspicion of outbreak response teams, including researchers. Distinctly American concepts such as FDA licensure and the animal rule are unlikely to mean much to these populations. Some degree of demonstrated efficacy in humans will likely be required to alleviate their suspicions. Furthermore, even in the United States, licensure based on the animal rule alone may not bring a sufficient degree of comfort to allow widespread use, even of a marketed product. Consequently, a strategy to translate discoveries from the laboratory to the field must be considered and take into account the challenges of conducting high quality and ethically sound research in some of the world's poorest nations.

4.1. Prerequisites

Despite the sense of urgency to intervene when faced with the devastating social and economic impacts of filovirus outbreaks in Africa, no compromises can be made on safety. Therapies and vaccines that show promise in animal models must first progress to Phases I and II clinical trials, which might include workers in high-containment laboratories and members of outbreak response teams. However, given the overwhelming human suffering associated with filovirus outbreaks, we must explore ways of safely expediting promising therapies and preventive strategies to compassionate use.

4.2. Identifying the countries and regions where filovirus clinical research could occur

Precisely when and where the next filovirus outbreak will occur cannot be predicted. However, past experience and our growing understanding of the ecology of filoviruses and their possible reservoirs can help us make some educated guesses (Peterson et al., 2004a,b; Leroy et al., 2005; Bausch et al., 2006; Pourrut et al., 2007; Swanepoel et al., 2007; Towner et al., 2007). Only Ebola Zaire and Sudan virus and Marburg virus, all found in Central Africa, have been associated with large outbreaks. Since the first discovery of a filovirus in 1967, the vast majority of cases have been observed in five countries: Gabon, the Republic of the Congo, the Democratic Republic of the Congo, Uganda, and Sudan (Table 1 and Fig. 1). Although numerous cases of EHF have also been seen in southern Sudan, that country is excluded from further consideration here due to the ongoing civil unrest which would presently constitute an insurmountable barrier to international research. Furthermore, relative "hot spots" can be identified within most of these countries where repeated transmission has occurred (Fig. 1). Major hospitals within these areas could be targeted for development as clinical research centers for FHF.

4.3. Assembling the partners and devising a plan

To date, field research during outbreaks of FHF has been almost entirely *ad hoc*, shaped by scientists once they hit the ground and based on available clinical samples. While this approach has led to considerable accumulation of knowledge on filovirus transmission and control, logistic, ethical, and legal considerations preclude its application to clinical research. Rather, a successful clinical research plan for the filoviruses will require considerable advanced planning. One approach would be to convene a filovirus clinical research working group, coordinated by the World Health Organization (WHO) and consisting

of key ministry of health representatives, clinicians, researchers, lawyers, and support personnel from the target regions in Africa, in addition to other international experts on filovirus research and clinical management, specialists in research ethics, logistical and legal support personnel from WHO, and representatives from any pharmaceutical companies potentially involved.

The working group would determine what clinical research questions regarding the filoviruses are most urgent and logistically amenable and devise detailed research protocols for each, taking into account the sometimes marked differences in social and official attitudes towards health and governmental programs between African regions. Because of the great distances involved, the approach may require developing mobile research teams and units. An essential element should be that all interventions be conducted in a manner that permits determination of their efficacy, in the setting of a controlled trial when logistically and ethically possible. Plans should include not only the development of experimental designs, but also of the logistical steps required to implement the necessary physical and administrative infrastructure in each region, including specifically identified personnel. The overall research aims set out by the working group should be unified, essentially constituting a multi-center trial, although the plan for implementation would be tailored to the specifics of each country. The integration of African scientists and clinicians into each step of the developing research program will be vital to its success, along with a long-term plan to retain them, most likely entailing integration of their activities into the day-to-day efforts oriented toward more common endemic diseases.

4.4. Establishing the necessary physical and administrative infrastructure

Considerable work will be required to establish the necessary infrastructure for quality clinical research. Attention must first be paid to developing an avenue for effective communication with the communities potentially involved. A successful clinical research program can only be established if the communities view themselves as equal partners and trust that the program is designed to work on their behalf, even if the benefit might not be immediate. At every step, careful attention must be paid to thoroughly communicate the research aims and realistic expected benefits to local populations likely to be involved, as the consequences of even the appearance that outsiders are experimenting on vulnerable populations would be disastrous. Some degree of formative social science research may be required to help understand and close the cultural and communication gaps likely to exist between the researchers and the subjects.

Once solid communication and partnership with the community are established, attention can be turned to more physical matters. The transfer of appropriate technology, including the means for laboratory diagnoses and clinical monitoring, is an obvious prerequisite. Installation of fixed equipment would be the most beneficial for the targeted centers in the long-term, but transient provision of portable point-of-care instruments could also be envisioned. In some countries, existing research institutions with technical infrastructure and expertise in the filoviruses could be valuable partners, such as the Centre International de Recherches Médicales de Franceville in Gabon and the Uganda Viral Research Institute in Uganda. The international organization Médecins Sans Frontières, which is already deeply involved in response to filovirus outbreaks, may also serve as an important partner, especially with regard to provision of patient-care (Jeffs et al., 2007). Various government and academic institutions engaged in filovirus research and with programs in sub-Saharan Africa could also be important partners. In some regions research infrastructure being established for other diseases, in particular HIV/AIDs, could also be expanded to encompass work on FHF.

Perhaps even more challenging than the establishment of the physical infrastructure will be the recruitment, training, and maintenance of the required personnel. Clinical investigation teams composed of both local healthcare workers and expatriate technical advisors will need to be established and trained in advance, ready to move into action with pre-scripted research protocols, data collection forms (some of which have already been devised (Colebunders et al., 2007), and culturally appropriate methods of informed consent that have already been approved through an ethical review process in each affected country. This may require creation of institutional review boards in some sub-Saharan countries and research centers. Because of the sporadic nature of filovirus outbreaks, protocols may need to be reviewed, updated, and reapproved at regular intervals.

4.5. Research areas for consideration

Initial research initiatives do not necessarily have to focus on the testing of experimental products (and probably should not). Numerous presently approved therapeutic strategies could be evaluated based on sound scientific evidence of the pathogenesis of FHF and the treatment of septic shock. Although controlled trials would be ideal, this may not be ethically possible when considering the use of many already approved products. Nevertheless, this should not constitute an impediment to their use and careful monitoring, using historical controls when possible. Considerable benefit to the patient, as well as increased knowledge about clinical management of FHF, may be gained by observational studies.

The most fundamental of questions and a logical place to start is to assess the impact of aggressive supportive care. Although no controlled trials on various types of routine interventions have been conducted, it is interesting to note that the case-fatality rate of the only human filovirus outbreak (excluding isolated cases) to occur in an area where aggressive supportive care was routinely possible – Marburg hemorrhagic fever in Germany and Yugoslavia in 1967 – was 22%, compared to 87% for all other large outbreaks in more remote and undeveloped areas of sub-Saharan Africa (Anon., 2005; Bausch et al., 2006). Whether this difference can truly be attributed to the quality of care or is influenced by differences in virus strain, route and dose of infection, underlying prevalence of immunodeficiency and co-morbid illnesses, different intensity of surveillance and ability to detect mild cases, or genetic susceptibility in the populations under observation in Europe and Africa is unknown, but certainly merits study.

Specific areas of clinical study might include assessment of the efficacy of early goal-directed therapy for hemodynamic management (Rivers et al., 2001) which has been shown efficacious in studies on septic shock, the optimal composition of intravenous fluids to be used in resuscitation (Brummel-Ziedins et al., 2006), the use of blood products and pressor agents, antibiotic prophylaxis of secondary infection, and the optimal management of the pregnant patient. The efficacy of activated protein C, which is already FDA-approved, might be another area of study, although the finding of bleeding, sometimes severe, as an adverse effect warrants caution (Bernard et al., 2001). Trials of experimental compounds, such as antisense and siRNA, on a compassionate use basis could also be considered for severe cases not responding to maximal supportive care. However, given the highly charged atmosphere of most filovirus outbreaks, extra care must be made to assure that the patient and the community understand the experimental nature of these products in this context. The risk is that communities may misinterpret and overestimate the potential benefit of compassionate use products, and thus be suspicious of accepting future therapies that may have more potential or even proven efficacy.

Another possible area for early clinical research is participation of African countries in Phase I and II testing of promising therapies and vaccines. Since therapies and vaccines for the FHFs will be most needed in sub-Saharan Africa, it will be important that these populations are represented in the safety testing. These studies would also have the advantage of being performed in a non-emergent setting, giving time to educate and inform the local population about the problem and nature of the research as well as valuable training in a relatively stress-free setting for investigators new to the field.

The most rigorous and potentially serious clinical research, Phase III clinical trials, would occur only in the last stages of this proposed strategy, with personnel and infrastructure now readied through their experience in the aforementioned studies. The relatively short duration of most outbreaks of FHF will require swift action, particularly for vaccine trials. Otherwise, ongoing outbreak response efforts, which must be implemented immediately, may limit transmission to a point that assessment of the vaccine's protective efficacy would be difficult. Obviously, the pharmaceutical maker of any new product to be tested will be a key participant at this stage. This will likely require dealing with a complex medico-legal environment in order for a maker to agree to use of their drug. Considerable concerns can be anticipated over both liability and the reputation of the company should any adverse events occur. Although this could be a considerable impediment to pharmaceutical company involvement, it should be emphasized that, if planned and executed appropriately, the company's reputation could stand to gain by taking humanitarian action on behalf of impoverished populations in sub-Saharan Africa in their time of need.

The research environment established through the above studies will also provide an important opportunity to explore the pathogenesis of FHF, comparing data from humans from those collected in the many studies using animal models. Important questions to be addressed include the relative importance of humoral versus cellular immunity in recovery, tropism and timing of infection of various tissues, the precise nature of the inflammatory mediators or inhibitors circulating in FHF and their mechanism in producing disease or recovery, elucidating the pathophysiologic hemodynamic profiles, identifying prognostic indicators, and exploring genetic determinants of infection and severe disease.

4.6. Data analysis and statistical power

In addition to the various impediments described above, the relatively small numbers of cases noted in most filovirus outbreaks is often been cited as an insurmountable barrier to Phase III efficacy trials (Gibbs, 2004). However, the high case-fatality rates associated with FHF may make up for the small sample size; a randomized placebo-controlled trial of a treatment expected to result in a two-fold reduction in case-fatality rate, say from 80% to 40%, would have 90% power to detect this difference (2-tailed $p < 0.05$) with the enrollment of just 30 patients in each group (total $n = 60$ patients) (Bausch et al., 2007a). Given the increasing frequency and case counts of filovirus outbreaks in recent years (Table 1), this sample population certainly seems achievable. Of course, if more aggressive supportive care can be routinely implemented and decreases the baseline case-fatality rate, greater numbers of patients would be required (but a good problem to have). The multi-center approach described above should maximize case enrollment. Historical controls may also be employed, especially for the various species of ebolavirus, for which case-fatality rates have been relatively consistent over the years. Lastly, as mentioned above, given how little we presently know about filovirus clinical syndromes, even detailed systematic observations will certainly considerably expand our knowledge base even if they do not always allow for rigorous statistical analysis.

5. Conclusions

Filoviruses continue to plague populations in Central Africa, with increasing frequency and size of outbreaks in the last decade. Meanwhile, industrialized countries have conducted intensive laboratory-based research that is starting to produce promising therapies and vaccines, bringing the issue of clinical trials to the forefront. A successful clinical research program for filoviruses will likely only be established through concerted advanced planning and the cooperation of a broad array of partners. We propose a multi-center graduated program in which the original focus would be on establishing the necessary physical and administrative infrastructure and human resources to improve and study the impact of aggressive supportive care, followed by trials of various already-licensed therapies for septic shock and coagulopathy, and ultimately progressing to Phase II and III clinical trials of new therapies and vaccines.

This or any approach to patient-oriented research on the filoviruses will require navigating a complex array of scientific, logistical, financial, and legal challenges. The breadth and complexity inherent in establishing such a program can only be briefly touched upon in a single manuscript such as this, and

our proposed strategy may be only one of various worthwhile approaches. This communication is intended to spur discussion and action on this important topic, and will have served its purpose if a clinical research program on FHF can ultimately be established, regardless of whether the ultimate program conforms exactly to the strategy advocated here.

Despite the seeming immensity of the obstacles, sufficient field experience has been garnered in recent years that could, if the political will is there, be synthesized into a concerted prospective clinical research program. In fact, there is already precedent for success, such as the WHO-coordinated Mano River Union Lassa Fever Network in Sierra Leone, Liberia, and Guinea, where progress is being made in developing infrastructure and international collaboration for research on a VHF and Category A Select Agent of disease (Lassa fever) in one of the worlds' poorest, and until recently war-torn, regions (Khan et al., 2008). Establishing a clinical research program for the filoviruses holds the promise of finally having realistic therapeutic and preventive options for one of the most dangerous diseases in the world. Furthermore, the knowledge acquired through the process would likely have significant benefit for the treatment of other VHFs. Lastly, and perhaps of still greater importance, the research infrastructure created could be applied to the study and control of a broad array of diseases endemic in sub-Saharan Africa, including HIV/AIDS and malaria, as well as prepare African nations for the intrusion of emerging threats such as avian influenza.

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