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New opportunities for field research on the pathogenesis and treatment of Lassa fever

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Abstract

Unlike many viral hemorrhagic fevers (VHFs), Lassa fever (LF) is not a rare disease that emerges only as sporadic cases or in outbreak form. Although surveillance is inadequate to determine the true incidence, up to 300,000 infections and 5000 deaths from LF are estimated to occur yearly. The highest incidence is in the "Mano River Union (MRU) countries" of Sierra Leone, Liberia, and Guinea. Although civil unrest in this region over the past two decades has impeded capacity building and research, new-found peace in recent years presents new opportunities. In 2004, the Mano River Union Lassa Fever Network (MRU LFN) was established to assist MRU countries in the development of national and regional surveillance, diagnosis, treatment, control, and prevention of LF. Here, we review the present literature on treatment and pathogenesis of LF and outline priorities for future research in the field made possible by the improved research capacity of the MRU LFN.

Keywords: Lassa virus; Lassa fever; Arenavirus; Viral hemorrhagic fever; West Africa; Antiviral therapy; Ribavirin; Pathogenesis

1. Introduction

Lassa fever (LF) is an acute and sometimes severe viral hemorrhagic illness caused by Lassa virus (LASV), a member of the family *Arenaviridae* (Enria et al., 2006). LF is endemic in parts of West Africa, where up to 300,000 cases and 5000 deaths occur yearly (McCormick et al., 1987b). Humans contract LF primarily through contact with contaminated excreta of rodents of the genus *Mastomys*, which is the natural reservoir (Monath et al., 1974b; McCormick et al., 1987b). Secondary transmission of LASV between humans occurs through direct contact with infected blood or bodily secretions (Enria et al., 2006). Nosocomial transmission and outbreaks have been described in healthcare facilities in endemic areas (Carey et al., 1972; Monath et al., 1973; Fisher-Hoch et al., 1995; WHO, 2005).

2. History of research on Lassa fever in West Africa

Although LF was first recognized in Nigeria in 1969 (Buckley and Casals, 1970; Frame et al., 1970; Troup et al., 1970), field studies in the wake of hospital outbreaks soon revealed the MRU countries of West Africa (Sierra Leone, Liberia, and Guinea) to have the highest incidence of the disease (Monath et al., 1973, 1974a; Fraser et al., 1974; Knobloch et al., 1982; McCormick et al., 1987a,b; Lukashevich et al., 1993; Bausch et al., 2001) (Fig. 1). The problem was particularly severe in eastern Sierra Leone, prompting the Centers for Disease Control and Prevention (CDC) to establish a laboratory and research and control program there in 1976, which subsequently provided the majority of our present-day scientific knowledge on LF (McCormick

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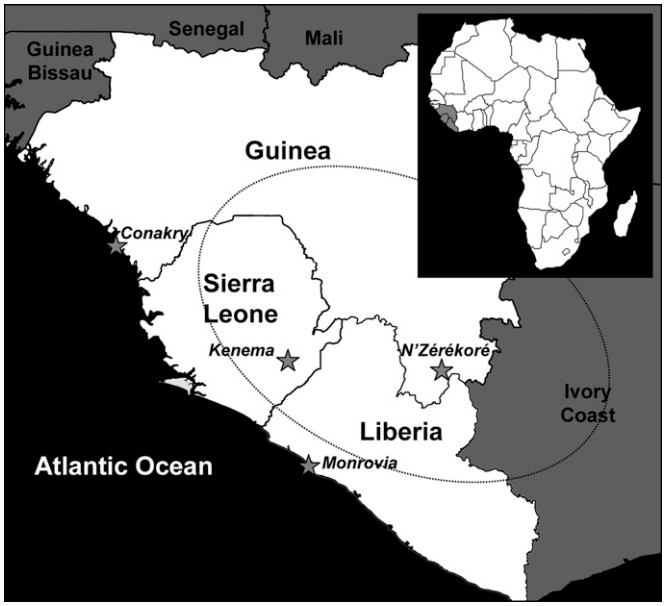

Fig. 1. Map of the Mano River Union countries (Sierra Leone, Guinea, and Liberia). The approximate known endemic area for Lassa fever is shown by the dotted oval. Sites of the four laboratories included in the Mano River Union Lassa Fever Network are indicated by stars and consist of the Kenema Government Hospital Lassa Laboratory in Kenema, Sierra Leone; the Central Public Health Laboratory Service in Monrovia, Liberia; the Program on Hemorrhagic Fevers in Conakry, Guinea; and the International Center for Research on Tropical Infections in N'Zérékoré, Guinea.

et al., 1987b). The program was based at Kenema Government Hospital (KGH) in the heart of the LF endemic area and included the establishment of a treatment ward and diagnostic laboratory (Bausch et al., 2004).

Civil war in Sierra Leone forced suspension of the CDC program in 1993, with subsequent transfer of the public health aspects to the relief organization Merlin (http://www.merlin.org.uk/) in 1996. The laboratory was not continued. Civil war also scuttled what had been an active research program in neighboring Liberia during this era (Mertens et al., 1973; Monath et al., 1973; Bloch, 1978; Frame et al., 1979, 1984a,b; Knobloch et al., 1980, 1982; Monson et al., 1984, 1987; Yalley-Ogunro et al., 1984; Van der Waals et al., 1986; Frame, 1989). Research on LF by various investigators has continued in neighboring Guinea (Lukashevich et al., 1993; ter Meulen et al., 1996, 1998; Bausch et al., 2001; Demby et al., 2001; Fair et al., 2007; Fichet-Calvet et al., 2007), but the apparent lower incidence of human disease (for unclear reasons) in that country has precluded extensive investigations on pathogenesis, clinical disease, and treatment. In fact, no studies have been published on treatment interventions for LF in the last 20 years.

3. Pathogenesis

The history of civil unrest and undeveloped biomedical infrastructure in the endemic area for LF, the relative danger of conducting full post-mortem exams on the bodies of patients who died of the disease, and certain African cultural taboos on manipulation of corpses have prevented extensive study of the pathogenesis and pathology of LF in humans. Our present understanding is thus based on the limited data from humans (Ikerionwu et al., 1978; Walker et al., 1982) combined with cautious extrapolation from extensive observations made in the excellent model of LF in non-human primates (Gowen and Holbrook, 2008).

The pathogenesis of LF appears to be related to unchecked viremia (Johnson et al., 1987). LASV is cleared rapidly in survivors. Cell mediated immunity appears to be the most important arm in recovery (Jahrling et al., 1985a; Peters et al., 1987). The humoral response often lags, with neutralizing antibodies typically appearing after recovery in survivors and not at all in most fatal cases (Johnson et al., 1987; Bausch et al., 2000). LASV infection probably results in life-long immunity, at least against severe disease, although this question has not been extensively studied in humans (McCormick et al., 1987b).

Microvascular instability and impaired hemostasis are the pathophysiologic hallmarks of LF and the VHFs (Enria et al., 2006). Contrary to popular thought, mortality does not usually result from exsanguination or direct virus-induced necrosis, although mild-to-moderate necrosis may be noted, especially in the liver and spleen (Callis et al., 1982; Walker et al., 1982). Rather, severe disease appears to result from the interaction of LASV with macrophages and dendritic cells, either directly or indirectly via soluble mediators, resulting in a process akin to septic shock, with activation of a host of inflammatory and vasoactive mediators leading to cellular dysfunction, insufficient effective circulating intravascular volume, and multi-organ system failure (Liu et al., 1986; Peters et al., 1987; Roberts et al., 1989; Guo et al., 1992, 1993; Qian et al., 1992; Aronson et al., 1995; Fennewald et al., 2002). One small study of LF in humans, however, found a lack of stimulation of various cytokines to correlate with a poor outcome (Mahanty et al., 2001). Data from animal models suggest that cardiac inotropy may be directly or indirectly inhibited, further impairing organ perfusion (Qian et al., 1994).

Dendritic cells and cells of the macrophage-monocyte line appear to be the primary initial sites of LASV replication after inoculation in animal models. After replication in the local tissues and regional lymph nodes, LASV is disseminated through lymph and blood monocytes to a wide variety of organ parenchyma and their associated mesothelial cell linings, including the liver, spleen, endothelium, lymph nodes, kidney, adrenal gland, pancreas, placenta, uterus, breast, and gonads (Jahrling et al., 1980; Callis et al., 1982; Walker et al., 1982). Inflammatory cell infiltrates are usually mild, consisting of a mix of mononuclear cells and neutrophils (Walker et al., 1982). Adrenal or pituitary gland necrosis with consequent vascular collapse has been postulated but not specifically demonstrated (Walker et al., 1982). Although mild thrombocytopenia typically occurs, hemorrhage appears to be primarily attributable to LASV-induced release of a soluble mediator impairing platelet aggregation (Cummins et al., 1989b). There is no evidence for a role of immune complexes, complement activation, or DIC as relevant pathogenic mechanisms in LF (Lange et al., 1985).

4. Clinical presentation and differential diagnosis

LASV infection may result in a spectrum of clinical effects ranging from asymptomatic to multi-organ system failure and death (McCormick et al., 1987a). Disease is seen in both sexes and all age groups (Webb et al., 1986). Reasons for the heterogeneity in severity are largely unknown, although differences in route and dose of infection, underlying comorbid illnesses, and genetic predisposition have been postulated.

The onset of illness is typically indolent, with non-specific signs and symptoms difficult to distinguish from a host of other febrile illnesses (Table 1). Typically, after an incubation period of about 10 days (range, 3–21 days), the patient notes the gradual onset of fever, headache, anorexia, malaise, and generalized weakness (Frame et al., 1970; Mertens et al., 1973; Monath et al., 1974a; Monson et al., 1984; McCormick et al., 1987a; Frame, 1989; Bausch et al., 2001). Within a few days these may be followed by sore throat with or without visible pharyngitis, retrosternal pain, tinnitus, conjunctival injection (typically *not* accompanied by itching, discharge, or rhinitis), nausea and vomiting, myalgia, arthralgia, lumbosacral pain, abdominal pain and tenderness, and diarrhea (Fig. 2A–D). Cervical lymph nodes may become enlarged. Tonsillar exudates sometimes noted at this stage have been known to prompt a misdiagnosis of streptococcal pharyngitis. A dry cough is sometimes noted, occasionally accompanied by an elevated respiratory rate and a few scattered rales, but prominent pulmonary symptoms or the presence of productive sputum early in the course of disease are uncommon. A maculopapular or petechial rash is usually noted over the thorax, face and arms in fair-skinned patients, but has not been noted in black Africans (McCormick et al., 1987a; Bausch et al., 2001) (Fig. 2F). Whether this difference reflects ease of observation or altered pathogenesis based on genetic makeup or previous exposure is uncertain. Jaundice is not typical of LF.

Table 1
Differential diagnosis of Lassa fever

Parasites
Malaria
Amebiasis
Giardiasis
African trypanosomiasis (acute phase)
Bacteria
Typhoid fever
Bacillary dysentery (including shigellosis, campylobacteriosis, salmonellosis, and enterohemorrhagic *Escherichia coli*)
Meningococcemia
Staphylococcemia
Septicemic plague
Tularemia
Streptococcal pharyngitis
Acute abdominal emergencies (appendicitis and peritonitis)
Anthrax (inhalation or gastrointestinal)
Psittacosis
Viruses
Influenza
Arbovirus infection (including dengue, yellow fever, and West Nile fever)
Viral hepatitis (including hepatitis A, B, and E, Epstein-Barr, and cytomegalovirus)
Measles
Rubella
Hemorrhagic or flat smallpox
Alphavirus infection (including chikungunya and o'nyong-nyong)
Other viral hemorrhagic fevers (including disease caused by Ebola, Marburg, Junin, Machupo, Guanarito, Sabiá, Flexal, Rift Valley fever, Crimean-Congo hemorrhagic fever, yellow fever, dengue, Omsk hemorrhagic fever, Kyasanur forest disease, and Alkhumra hemorrhagic fever viruses)
Spirochetes, Rickettsia, Ehrlichia, and Coxiella
Relapsing fever
Leptospirosis
Spotted fever group rickettsia (including Rocky Mountain spotted fever, Boutonneuse fever, African tick bite fever)
Typhus group rickettsia (including murine- and louse-borne typhus)
Q fever
Ehrlichiosis
Non-Infectious Etiologies
Thrombotic thrombocytopenic purpura

After 4–7 days of illness, a minority of patients progress to severe vascular instability which may be manifested as edema (especially of the face and neck), bleeding, hypotension, shock, and proteinuria (Enria et al., 2006) (Fig. 2D,E,G,H). Clinically discernible hemorrhage is seen in less than 20% of hospitalized cases (McCormick et al., 1987a; Bausch et al., 2001) and typically consists of a mild oozing from the nose, mouth, or, less frequently, the rectum. Central nervous system manifestations, including disorientation, gait anomalies, convulsions, hiccups, and coma may be noted in end-stage disease (McCormick et al., 1987a; Bausch et al., 2001). It is not clear whether these represent infection of the central nervous system by LASV, secondary immune-mediated effects, or nonspecific metabolic ones common to any critically ill patient (Solbrig and McCormick, 1991; Solbrig, 1993). Pleural and pericardial effusions are occasionally seen late in the course of the disease (Hirabayashi et al., 1988; Yanase et al., 1989).

Maternal and fetal mortality are elevated in pregnant women with LF, especially during the third trimester, when vaginal bleeding and spontaneous abortion usually occur, with fetal death rates approaching 100% (Monath et al., 1974b; Price et al., 1988). A "swollen baby syndrome" comprised of anasarca, abdominal distention, and bleeding with a high mortality has been described, but whether this is a general feature of LF in infants or related to other concomitant health risks or therapies specific to the area of study is not clear (Monson et al., 1987).

Death from LF usually occurs 10–14 days after symptom onset. Common indicators of a poor prognosis include shock, bleeding, neurological manifestations, high viremia (or surrogate measurements of antigen or genome copies), and levels of aspartate aminotransferase (AST >150 IU/l) (Frame et al., 1970; Johnson et al., 1987; McCormick et al., 1987a; Bausch et al., 2000). Although a broad range of case-fatality rates have been reported, about 20% is a good estimate in patients seeking medical treatment. Nigerian outbreaks have often been associated with a higher mortality rate than those in MRU countries. There is significant sequence heterogeneity in LASVs circulating in West Africa and sometimes even within the MRU

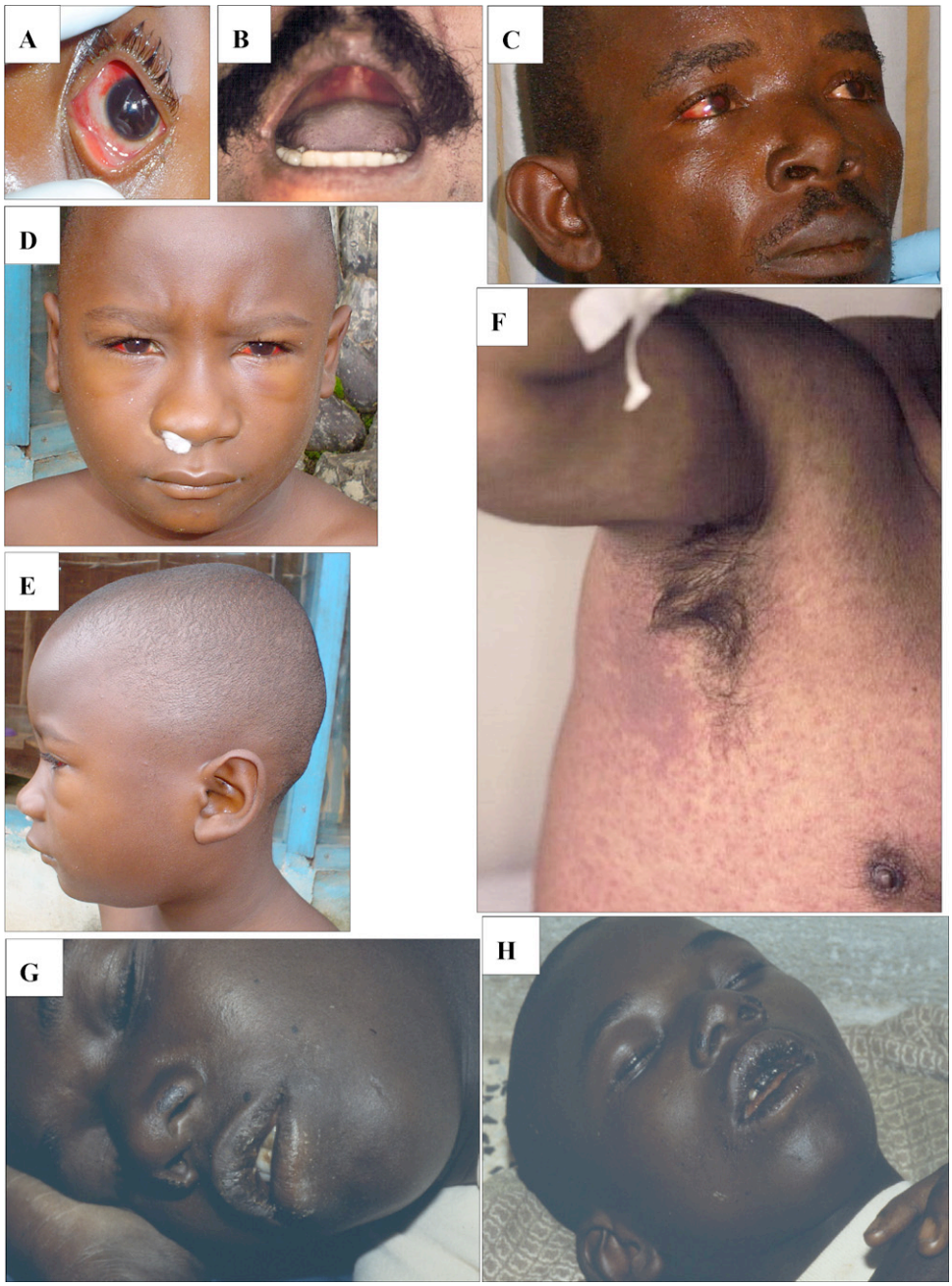

Fig. 2. Clinical manifestations of Lassa fever. (A) Severe conjunctival injection in a young boy. (B) Soft and hard palate erythema in an Indian soldier serving as a United Nations Peacekeeper in Sierra Leone. The patient was treated with intravenous ribavirin and survived. (C) Mild subconjunctival injection in an adult. (D and E) Severe subconjunctival hemorrhage, epistaxis (managed by the patient with a cotton plug in the nostril), and facial swelling in a young boy. (F) Maculopapular skin rash in the same soldier presented in image B. (G) Facial swelling in a teenage boy. (H) More severe oral mucosal bleeding in the same patient 12 h later. The patient presented very late to the hospital and, despite treatment with ribavirin, died shortly after this picture was taken. (Photos A, D, and E by Ibrahima Camera; B, C, and F–H by Daniel Bausch.)

countries, with evidence from animal models that some strains are more lethal (Jahrling et al., 1985b; Bowen et al., 2000). The impact of other comorbid conditions such as malnutrition, malaria, and human immunodeficiency virus infection, common in the impoverished rural regions where LF is endemic, is unknown.

Common clinical laboratory findings early in the course of LF include mild thrombocytopenia (not usually <100,000/μl), mild leucopenia with lymphopenia, moderate hemoconcentration, elevated blood urea nitrogen, and proteinuria (Mertens et al., 1973; Monath et al., 1974a; Monson et al., 1984; McCormick et al., 1987a; Fisher-Hoch et al., 1988; Frame, 1989; Schmitz et al., 2002). Severely ill patients are more likely to be thrombocytopenic, are usually lymphopenic, and may have leukocytosis with neutrophilia. Amylase and hepatic transaminase levels are also often elevated, with AST significantly greater than the alanine aminotransferase (ALT), suggesting that the source of these enzymes is not solely the liver, but probably a product of diffuse

tissue ischemia and damage (Johnson et al., 1987). A variety of nonspecific electrocardiographic changes have been reported (Cummins et al., 1989a). The few chest radiographs taken of patients with LF have generally correlated with the physical exam (Ketai et al., 2003).

Sensorineural deafness is the major chronic sequela of LF (Cummins et al., 1990). Although deafness has been reported to occur in as many as 25% of cases, this seems like an overestimate from experience in Kenema in recent years. Deafness typically presents during convalescence and is unassociated with the severity of the acute illness, level of viremia, or AST level, suggesting an immune-mediated injury (Cummins et al., 1990). It may be unilateral or bilateral, and is permanent in approximately two thirds of cases. Auditory patterns resemble idiopathic nerve deafness (Liao et al., 1992). Depression and cerebellar ataxia have been reported during recovery, but are relatively uncommon (Solbrig, 1993). LASV can often be isolated from the cerebrospinal fluid, where a mild-to-moderate leukocyte infiltration has also sometimes been noted, but a systematic correlation with any of the neurologic manifestations of the disease is not available (Johnson et al., 1987; Cummins et al., 1990; Solbrig and McCormick, 1991; Gunther et al., 2001).

5. Current drug treatment

Based on a single study published in 1986, the current drug treatment for LF is the guanosine analogue ribavirin, which has been shown to reduce the mortality of severe disease from 55 to 5% when administered intravenously (IV) within the first 6 days of illness (McCormick et al., 1986). This is an off-label indication for ribavirin in the United States. Oral ribavirin is also effective, although less so than the IV form, most likely because the serum concentration achieved through oral administration is on the borderline of the mean inhibitory concentration of ribavirin for LASV (4–40 μM) (Jahrling et al., 1980; Connor et al., 1984; McCormick et al., 1986). Oral ribavirin has also frequently been advocated as post-exposure prophylaxis (Huggins et al., 1984; Johnson, 1986; McCormick et al., 1986; Anon., 1988; Huggins, 1989; Cummins, 1990; Johnson and Monath, 1990; Crowcroft, 2002; Bossi et al., 2004), although no data exist on its efficacy for this indication and guidelines vary on indications for use, dose, and duration of therapy (Anon., 1988; Holmes et al., 1990; Johnson and Monath, 1990; Haas et al., 2003; Bossi et al., 2004). Observational studies on oral ribavirin for post-exposure prophylaxis of LF suggest that adherence is low and minor adverse effects such as nausea and vomiting are frequent (Hadi et al., manuscript in preparation). The primary adverse effects of ribavirin are reversible hemolytic anemia and rigors when the drug is infused too rapidly IV (McCormick et al., 1986, Fisher-Hoch et al., 1992, Chapman et al., 1999, Haas et al., 2003). Although patent issues and high cost (~$1000 per patient when acquired from the patent holder) have historically severely limited availability of IV ribavirin, the patent is now expired and in 2007 the drug was added to the World Health Organization's (WHO) list of essential medicines, which should significantly lower its cost and improve its availability to populations in need in West Africa.

6. New opportunities for research: the Mano River Union Lassa Fever Network

Although Sierra Leone endured a grisly civil war for much of the past 2 decades, the war ended in 2002 and the country is now politically and economically stable and taking steps to rebuild. Similarly, Liberia's long civil war ended in 2003 and both countries have recently held elections with peaceful transitions of power. The new-found peace in the region offers an opportunity to reestablish the biomedical infrastructure, including that dedicated to research on LF.

Recognizing the continued threat that LF poses to the indigenous population of the MRU counties, as well as to visiting expatriates, in 2004 a diverse group of organizations, including MRU country governments, WHO, Tulane University, the United Nations, and the United States' Office of Foreign Disaster Assistance (OFDA) and the US Army Medical Research Institute of Infectious Diseases (USAMRIID) established the Mano River Union Lassa Fever Network (MRU LFN, http://www.sph.tulane.edu/ManoRiverLassa). The MRU LFN assists in the development of national and regional prevention and control strategies for LF and other dangerous diseases, including the enhancement of laboratory diagnostic capacity, and training in laboratory diagnosis, clinical management, and infection and environmental control. The MRU LFN program is coordinated by the BioRisk Reduction for Dangerous Pathogens team of WHO's Department of Epidemic and Pandemic Alert and Response, with Tulane University contracted as the principle implementing partner.

The cornerstone of the MRU LFN is enhancement of laboratory capacity. The emphasis on the laboratory was a conscious decision taking into account the very non-specific clinical presentation of LF that continually undermines efforts to establish surveillance and control programs and the extreme difficulty of sending diagnostic specimens for testing at laboratories outside the MRU region. Although diagnostics for LF have been intermittently performed at a few MRU country laboratories since the closure of the CDC program in Sierra Leone, a variety of methods were being used with little internal or external quality control. In view of this, governments and other organizations working in the region were obliged to send samples to one of the few laboratories capable of performing diagnostic testing for LF in Europe, South Africa, or the United States. With the inherent logistical delays, progressively increasing as the regulatory environment for Select Agents becomes increasing strict, samples inevitably arrived at their destination laboratories and results were rendered too late to be of practical use in patient care or even contact tracing (despite usually excellent turn-around times at the laboratory), in most cases months after the index case was seen.

Rather than start completely *de novo*, the MRU LFN builds on four pre-existing laboratories in the MRU countries: the KGH Lassa Laboratory in Kenema; the Central Public Health Laboratory Service in Monrovia, Liberia; the Program on Hemorrhagic Fevers in Conakry, Guinea; and the International Center for Research on Tropical Infections in N'Zérékoré, Guinea (Fig. 1). The laboratories are at different stages of development. It is not

Fig. 3. The Kenema Government Hospital Laboratory. The laboratory is comprised of an approximately 5500 ft^2 building (center) divided into a general clinical laboratory for routine diagnostics and a 700 ft^2 specialized biosafety level-3 suite for manipulation of samples from suspected cases of Lassa fever. Three rectangular solar panels that can faintly be seen on the roof provide essential power for refrigerators, freezers and other essential instruments. The smaller structure in the foreground is constructed from pre-fabricated office containers donated by the United Nations, some of which have been subsequently refitted to serve as PCR clean rooms. The existing Kenema Government Hospital Lassa Ward is behind the buildings up the hill to the left, not visible in this view. (Photo by Joseph Fair.)

necessarily intended that all four laboratories will provide testing for LF, but rather that they will form a collaborative network in which the repertoire of tests performed at each laboratory will be based on the country's and the region's public health and research needs.

Given that the incidence of LF is highest in Sierra Leone, the initial focus of the MRU LFN program has been the laboratory at KGH. The laboratory is located on the grounds of the hospital, but in a stand-alone building constructed in 2005 with support from Merlin, the United Nations Mission in Sierra Leone, OFDA, WHO, Tulane University, and the Sierra Leone Ministry of Health and Sanitation (MOHS). The building has approximately 5500 ft^2 of laboratory space divided into a general clinical laboratory for routine diagnostics and a 700 ft^2 specialized biosafety level (BSL)-3 suite for manipulation of samples from suspected cases of LF (Fig. 3). Access to the building and the BSL-3 suite is controlled. Samples are manipulated in class II biosafety cabinets by personnel wearing full personnel protective materials (gowns, gloves, and mask) (Fig. 4). Negative airflow is maintained. The laboratory possesses equipment and trained personnel for diagnostics using ELISA, real-time and conventional PCR (with separate PCR suites), and immunofluorescent antibody tests. For safety reasons, no cell culture is performed. The building is equipped with redundant power sources, including town power in Kenema, which is extremely sporadic, and 100 and 6 kV generators, the former used to power the entire laboratory and the latter essential equipment only. The cold storage consists of a −80 °C freezer connected to the town power/generator grid and solar powered −20 °C freezers and refrigerators. Established biosafety and biosecurity guidelines are maintained, with oversight by WHO and Tulane University through the MRU LFN.

At present, real-time PCR and ELISA (antigen and IgM and IgG antibody) for LF are being carried out at the KGH Lassa Laboratory. LASV antigens, monoclonal antibodies, and other reagents for ELISA are provided by USAMRIID following a published protocol (Bausch et al., 2000), with some modifications. In addition, through a National Institutes of Health (NIH) grant, Tulane University and various partners are developing recombinant diagnostic reagents for LASV and other arenaviruses that have shown promising preliminary results and may soon "release the bottleneck" imposed on LF research by the extremely limited availability of reagents. The necessary administrative infrastructure for clinical research is also being put into place in Sierra Leone; the government has an ethics

Fig. 4. Sample Processing in the Kenema Government Hospital Laboratory. Samples are manipulated in class II biosafety cabinets by personnel wearing full personnel protective materials. (Photo by Joseph Fair.)

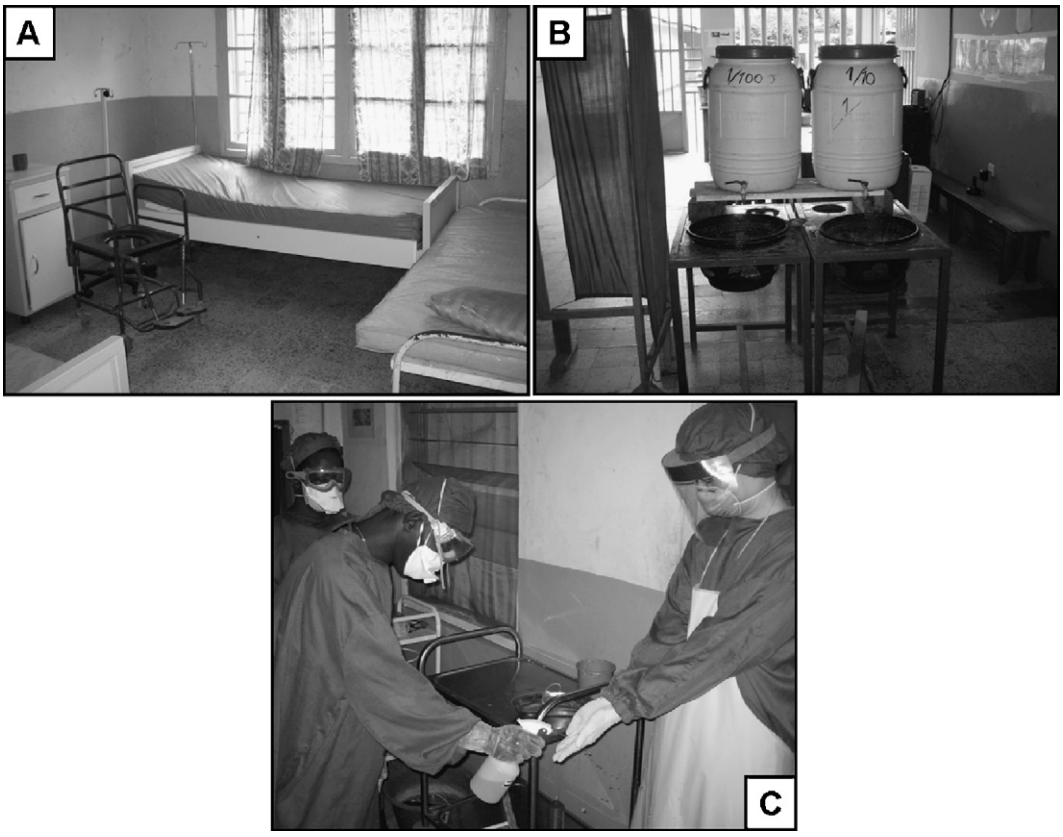

Fig. 5. The Kenema Government Hospital Lassa Ward. Unlike the new laboratory building, the ward is antiquated and in need of major repair or replacement. (A) Small multi-bed room without equipment for optimal medical care. (B) Decontamination station. (C) Decontaminating gloves after a patient exam. (Photos A and B by Christiane Hadi; C by Daniel Bausch.)

committee to review and approve research protocols and has received Federal Wide Assurance from the US Department of Health and Human Services. Furthermore, KGH has recently completed registration with the NIH, making the center eligible to apply for NIH funding.

7. Research priorities for treatment and pathogenesis

The enhanced physical and organizational infrastructure provided by the MRU LFN and KGH Lassa Laboratory, and especially the implementation of real-time laboratory diagnostics, offer tremendous potential for research on LF. Key areas of investigation include the development and testing of new diagnostic techniques (such as the aforementioned recombinant-based assays); a more complete understanding of the clinical presentation, pathogenesis, correlates of immunity, and natural history of LASV transmission; and the development and testing of new treatments and vaccines. Some of the priorities and opportunities for research on the treatment of LF include:

7.1. A better understanding of disease pathogenesis and determinants of severity

A deeper understanding of the pathogenesis of LF is essential to identifying targets for optimal use of present and future therapeutic interventions. Studies should entail elaboration of the dynamics of viremia, cellular and humoral response, and circulation of cytokines and other inflammatory and vasoactive mediators. These measurements should be performed in tandem and correlated with careful clinical observations, pulse and blood pressure monitoring, and clinical laboratory examinations. The knowledge gained could have direct and immediate implications for patient management. For example, if the decrease in cardiac index and increase in peripheral vascular resistance noted in animal models of LF can be confirmed in humans, this would favor the early use of inotropes and vasoconstrictors to maintain organ perfusion, rather than aggressive volume repletion that might result in fluid overload.

Given the extreme variability in severity of LF, identifying profiles of patients who will ultimately develop severe disease is also a goal. Presently known prognostic indicators, as detailed above, are all essentially downstream markers measured after the disease is well under way. This research would include exploration of the potential role of specific human host genotypes and previous infection in the severity of disease. The value of the AST level as a prognostic indicator should also be confirmed, as it provides an almost unparalleled opportunity to evaluate candidate antiviral compounds and strategies in studies of relatively few patients.

7.2. Efficacy of general supportive measures

Ironically enough, no studies exist on the efficacy of modern supportive measures, such as aggressive rehydration, the admin-

istration of replacement blood products and pressor agents, and intensive monitoring of hemodynamics and serum electrolytes, primarily because the medical and research infrastructure to administer and monitor such interventions have never been available in areas of the world where appreciable numbers of LF cases are seen. Interestingly, the case fatality of the only significant outbreak of VHF to occur in a setting where more advanced care was possible – Marburg hemorrhagic fever in Germany and Yugoslavia in 1967 – was 22%, compared to 80–90% for other large outbreaks of the same disease in remote and undeveloped areas of sub-Saharan Africa (Anon., 2005; Bausch et al., 2006). Whether similar dramatic improvements in the mortality rate are achievable with aggressive supportive care for LF remains to be seen. Specific questions for study might include the efficacy of early goal-directed therapy for hemodynamic management (Rivers et al., 2001) and intensive insulin therapy (Van den Berghe et al., 2001), both of which have been shown efficacious in studies on septic shock, the optimal IV fluid to be used in resuscitation (Brummel-Ziedins et al., 2006), and the optimal management of the pregnant patient with LF.

7.3. Optimization of ribavirin therapy

Although data and experience support the efficacy of ribavirin for LF, various questions persist on its optimal use. The 10-day IV regimen that has become the standard of care derives from extrapolation from animal studies (Connor et al., 1984; McCormick et al., 1986). Whether shorter durations of therapy, which would diminish costs and adverse effects, would be equally efficacious is unknown. Although ribavirin-induced anemia is usually not profound, especially with the short duration of therapy indicated for LF, any measure exacerbating blood loss is obviously of concern in patients with VHF, especially considering that baseline hematocrits may be low in impoverished populations in MRU countries where LF is endemic.

Another important question is if and when it would be safe to convert from IV to oral ribavirin as a patient's condition stabilizes. In the absence of this knowledge, and given the finding of diminished efficacy of oral ribavirin, physicians usually feel obligated to give a full 10-day course of the IV drug, requiring costly and inconvenient prolongation of hospitalization even when patients feel well. Head-to-head equivalency trials might compare various IV regimens and include an arm with conversion to oral therapy after stabilization.

Although evaluation of the efficacy of oral ribavirin as post-exposure prophylaxis is also a theoretical research priority, the low secondary attack rate of LF makes it unlikely that enough subjects could ever be enrolled for a conclusive study of this question to be performed, even in Sierra Leone (Fisher-Hoch et al., 1985).

7.4. Testing of new anti-arenavirus drugs

Although ribavirin is efficacious, fatalities are still noted with its use. There is thus certainly room for new treatments that could replace or supplement ribavirin. Indeed, various new therapeutics are being explored for LASV and other arenaviruses, including small interfering RNAs (Sanchez et al., 2005, Muller and Gunther, 2007) and novel nucleoside analogs (Uckun et al., 2004, 2005). Furthermore, assessment of transcriptome profiles in monkey models of arenavirus infection has shown that transcription of multiple genes is strongly upregulated or downregulated before the viremic stage and sustained throughout the first few days of disease (Djavani et al., 2007). Many of these genes are the targets of FDA-approved drugs, although the clinical significance of this remains unknown. Oral drugs that could achieve the target MIC for LASV would constitute a significant advancement, either for use as primary treatment, conversion from the IV form after a patient's clinical condition stabilizes, or as post-exposure prophylaxis. Such a drug could also be considered for inclusion in the U.S. Strategic National Stockpile for use in public health emergencies, including bioterrorist attacks.

7.5. Testing of non-arenavirus-specific drugs

Although new anti-arenavirus therapies may hold promise for the future, few of these products have reached or are presently ready for clinical phases of testing. However, various non-arenavirus-specific therapeutic strategies could soon be considered for careful clinical use and study. Consensus interferon-α (interferon alfacon-1) is FDA approved for use in humans (although treatment of LF would be off-label) and has been shown to have a synergistic effect with ribavirin to diminish mortality and disease severity in a hamster model of arenavirus infection (Gowen et al., 2006). In vitro studies suggest interferon to play a role in controlling LASV infection (Asper et al., 2004; Baize et al., 2006), although this conclusion has not always been supported from animal studies (Peters et al., 1989). Human trials on the efficacy of ribavirin with and without interferon alfacon-1 could be undertaken, with the goal of not only diminishing mortality and disease severity, but also minimizing the adverse effects of ribavirin, since the addition of interferon might allow the use of lower doses.

A growing body of literature suggests that disturbances in the procoagulant-anticoagulant balance play an important role in the mediation of septic shock, including in the VHFs. Another compound that could be considered for clinical trials in LF is recombinant activated protein C, an FDA-approved drug which has been found to diminish mortality in severe septic shock through modulation of the interlinked inflammatory and coagulation cascades (Bernard et al., 2001), although some controversy over its efficacy persists (Feistritzer and Wiedermann, 2007). At first glance, the major adverse effect of activated protein C – serious bleeding (including intracranial hemorrhage), which has been reported in up to 5% of the patients (Bernard et al., 2003; Vincent et al., 2005) – would seem to contraindicate its use in VHF. However, the mechanism of activated protein C may not, in fact, be via direct anticoagulation, but rather through modulation of inflammation. Conceivably, early use could mitigate the pathogenic processes in VHF that ultimately result in hemorrhage, with no additional risk of bleeding due to the drug itself (Vincent et al., 2005). Furthermore, the infrequency and mildness of bleeding in LF relative to most other VHFs

(McCormick et al., 1987a; Bausch et al., 2001) might make it a logical candidate for trials with this drug.

A number of new strategies to develop a vaccine for LF are also being explored, including the use of recombinant and attenuated viruses (Geisbert et al., 2005; Lukashevich et al., 2005; Bergthaler et al., 2006; Bredenbeek et al., 2006; Carrion et al., 2007), DNA vaccines (Rodriguez-Carreno et al., 2005), and alphavirus replicons (Pushko et al., 2001). All of these vaccines are still in pre-clinical phases of development but the MRU LFN will constitute an invaluable resource if and when these products are ready for efficacy trails in humans.

7.6. Collaboration between field and basic research scientists

The infrastructure of the MRU LFN offers tremendous potential for collaborative studies with basic scientists making advances in the laboratory, in particular, the recent development of reverse genetics approaches to LASV (Hass et al., 2004). Reverse genetics could also serve as a valuable logistical tool to safely and legally bypass the regulatory obstacles involved in shipping Select Agents to outside collaborators; non-infectious LASV RNA could be shipped from MRU countries to basic science laboratories registered to work with Select Agents, where infectious recombinant LASV could be reconstituted under BSL-4 conditions. The selection of qualified collaborating laboratories would be by the MRU LFN scientific advisory committee, which consists of members from the involved laboratories, and advisors from MRU country governments, WHO, Tulane University, and other relevant international authorities. The detailed procurement (i.e. human or rodent) and clinical history available on each RNA sequence sent from West Africa, the ability to manipulate the LASV make-up through the reverse genetics system, and the existence of excellent animal models would afford tremendous potential for the understanding of the pathogenesis of LF, as well as for the development of novel antiviral strategies and vaccines. The most efficacious and safest therapeutics identified through laboratory research could then be returned to West Africa for clinical trials implemented and overseen by the MRU LFN.

8. Current challenges to research development in Kenema

KGH maintains a full-time 25-bed ward and staff dedicated to the care of patients with LF. A case-definition for LF is used to aid diagnosis, developed largely through experience at the site (Table 2). Standard guidelines for the isolation and management of patients with VHFs are maintained (Anon., 1995; CDC and WHO, 1998; Bausch, 2007). However, in contrast to the new laboratory, the ward is antiquated and lacking in equipment for optimal modern medical care and clinical research (Fig. 5). The electrical supply is inconsistent at best and without back-up, almost all rooms are multi-patient, and no advanced hemodynamic monitoring or mechanical ventilation is available. Construction was recently begun on a new ward with external funding, but logistical and financial problems between the donor and the contractor eventually led to abandonment of the project. A new donor is presently being sought. Support is also needed to stabilize the supply of personal protective materials and to assure proper infection control practices in both the Lassa ward and KGH in general, where a nosocomial outbreak of LF in the pediatric ward was reported in 2004, most likely attributable to a LASV-contaminated multi-dose vial of antibiotics/saline and reuse of needles and syringes (WHO, 2005). Toward this goal, the MRU LFN has conducted a series of training workshops on infection control and clinical management of patients with VHFs, and Tulane University has also recently sponsored a consultant to assess infection control conditions at KGH, which has resulted in the first-ever creation of a position specifically assigned to infection control at that hospital.

Another major challenge to clinical research on LF in Kenema is case finding and patient access to the Lassa Ward. Since 2004, the number of admissions to the ward has consistently diminished, while the case-fatality has more than doubled (Table 3). Although the decreased number of cases might initially be interpreted as an indicator of successful disease prevention, the increasing case-fatality rate suggests otherwise. A more likely explanation is decreased surveillance and case identification after the 2004 departure from Sierra Leone of Merlin, who managed the KGH Lassa ward as well as an active outreach program for LF during and immediately after the civil war. In addition, Dr. Aniru Conteh, the Chief Medical Officer of the

Table 2
Case definition for a suspected case of Lassa fever at Kenema Government Hospital

√ Fever >38 °C for less than 3 weeks and
√ Absence of signs of local inflammation (i.e. the illness is systemic) and
√ Absence of a clinical response after 48 h of anti-malaria treatment and/or a broad-spectrum antibiotic and
√ Two major signs or one major sign and two minor signs described below
Major signs
Bleeding (including from the mouth, nose, rectum, or vagina)
Swollen neck or face
Conjunctivitis or subconjunctival hemorrhage
Spontaneous abortion
Petechial or hemorrhagic rash
New onset of tinnitus or altered hearing
Persistent hypotension
Elevated liver transaminases, especially AST > ALT
Known exposure to a person suspected to have Lassa fever
Minor signs
Headache
Sore throat
Vomiting
Diffuse abdominal pain/tenderness
Chest/retrosternal pain
Cough
Diarrhea
Generalized myalgia or arthralgia
Profuse weakness
Proteinuria
Leucopenia <4000/μl

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 3
Number of patients admitted and mortality in the Kenema Government Hospital Lassa Ward, 2004–2007[a]

Year	No. patients admitted	No. deaths	Case fatality (%)
2004	321	80	25
2005	136	35	26
2006	51	17	34
2007[a]	19	11	58

[a] Data are from the first 6 months of 2007.

Lassa Ward, tragically died of LF in 2004 after a needle-stick injury (Bausch et al., 2004). In recent years, patients admitted to the Lassa Ward, often after significant delays, have frequently commented that they assumed the ward to be defunct since the departure of Merlin and the death of Dr. Conteh. Consequently, it is likely that only the most severe and recognizable cases of LF are presently being noted and eventually sent to the hospital, often arriving too late to have much impact on the outcome. Consistent with this theory is the fact that the mean time from symptom onset to presentation for care at the KGH Lassa Ward has steadily risen from 8.4 days in 2005 to 10.9 days in 2007 (KGH Lassa Ward statistics). Economic and social hardships exacerbated by the departure of many relief organizations since the end of the war and the aforementioned suboptimal conditions of the existing KGH Lassa Ward no doubt contribute to failure of patients to present for care.

In addition to the obvious impact on patients' health, this situation has serious implications for the ability to conduct patient-centered research on LF in Kenema. The efficacy of any therapeutic measure can only be assessed if considerable numbers of patients present in a reasonable time frame to allow interventions. Recognizing this, the European Union has recently funded WHO and the MRU LFN to mount an intensive surveillance program for LF in eastern Sierra Leone, which will be implemented in concert with surveillance for other priority epidemic-prone diseases in the region, such as yellow fever, measles, and avian and human influenza. Research projects are also underway or planned on the circulation of flaviviruses in Guinea and surveillance for monkeypox in all three MRU countries.

Still other impediments to the development of clinical research in Sierra Leone and the MRU countries come in the form of lack of human resources. Few clinicians or administrative personnel in the region are trained or have experience with clinical research. Taking the first steps to remedy this, two members of the Sierra Leone MOHS recently attended an NIH-sponsored workshop on clinical trials in South Africa. Another problem is reliability of clinical laboratory testing at KGH and other MRU LFN laboratories and hospitals. Although diagnostics for LF at KGH are in place, only a limited panel of supportive clinical laboratory tests is available from the general hospital laboratory, generally with minimal internal or external quality control. Recognizing the importance of close laboratory monitoring of patients involved in clinical trials, the MRU LFN is planning enhanced training in clinical laboratory testing, both on-site as well as through travel of key laboratory personnel for intensive training overseas.

Lastly, we cannot forget that the MRU countries are among the poorest in the world and, in the case of Sierra Leone and Liberia, are slowly and cautiously emerging from years of failed government and civil war that have decimated their public health and biomedical infrastructure. With the return of peace to the region, there are many competing priorities. With recent elections, Sierra Leone and Liberia seem to be turning the corner. In contrast, Guinea, formerly the most stable of the three countries, was repeatedly rocked by nationwide strikes and protests in 2006 and 2007. Its future remains uncertain.

9. Future challenges and potential

In the last decade, industrialized countries' fears over bioterrorism have fueled research on diagnosis and treatment of LF and other Category A Select Agent diseases to an unprecedented extent (Schuler, 2005). Bolstered by excellent animal models for LF, this basic science research will eventually render products ready for clinical testing. In Sierra Leone and the other MRU countries, the renewed political stability, hyper-endemicity of LF, and enhanced physical and organizational infrastructure provided by the MRU LFN and affiliated projects constitute an unparalleled setting for prospective study of LF. Furthermore, new antiviral compounds and treatment strategies that succeed for LF may prove effective for the other arenaviruses. Nevertheless, a continued and concerted influx of appropriate funding and technology will be necessary to capitalize on this important and unique opportunity. The solid integration of African scientists and clinicians into each step of the developing research base will be vital to its success. Extreme caution must be made to provide thorough communication of the research aims and realistic expected benefits to West African populations that might be involved. If successful, the MRU LFN has the potential to protect persons around the globe from LF and other arenavirus diseases, whether acquired through natural or intentional transmission. Furthermore, the MRU LFN may serve as a model for broad regional cooperation to confront VHFs and other threats to health in sub-Saharan Africa and beyond.

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