

Moving Lassa Fever Research and Care Into the 21st Century

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(See the major article by McElroy et al on pages 1862–72.)

Lassa fever, a viral hemorrhagic disease, is a growing threat to public health in West Africa and beyond. While Ebola virus disease (EVD) recently captured the attention of the global community, Lassa fever arguably represents an even more concerning cause of viral hemorrhagic fever (VHF). Unlike EVD, which causes sporadic outbreaks, Lassa virus (LASV) is endemic in West Africa and is responsible for an estimated 300 000 infections and >5000 deaths annually—figures that are likely underestimated, given the challenge of collecting epidemiologic data due to civil conflict and limited clinical research infrastructure in endemic countries [1–3]. As a result, in a given 3 year period, Lassa fever kills more people than all the known outbreaks of EVD combined. Moreover, the World Health Organization (WHO) reports an upward trend in the incidence of Lassa fever this past year, perhaps as a consequence of climate change, with an increase in severity and case fatality rate [4]. Many of these deaths could be prevented with better diagnostics, supportive clinical care, and therapeutics [4, 5]. Furthermore, with >32 reported cases of Lassa fever imported into nonendemic countries—one-third of which were fatal—the significance of enhanced detection and management of this virus extends beyond West Africa

[6–10]. For these reasons, in 2016, the WHO released a research and development blueprint call to action that identified LASV as a “top priority emerging pathogen” likely to cause a severe outbreak in the near future and consequently urgently needing to be studied [11].

Despite being a major cause of infection in West Africa, Lassa fever remains underdiagnosed and understudied. It has been almost 50 years since LASV was first identified during a 1969 outbreak in Nigeria, but the mechanisms underlying its pathogenesis and the role that host and viral factors play remain unclear. With the notable exception of tenacious efforts by research groups at Kenema Government Hospital in Sierra Leone a Irrua Specialist Teaching Hospital in Nigeria, Lassa fever clinical research has been largely petrified. Limited studies from the 1980s suggest that uncontrolled viremia is highly predictive of disease severity [12]. Patients presenting with systemic viral loads <10³ median 50% tissue culture infective dose (TCID₅₀)/mL were 3.7 times more likely to survive than those admitted with higher levels [13]. Additionally, survival has also been linked to the induction of a LASV-specific cell-mediated immune response, rather than a humoral immune response, as the appearance of antibodies does not seem to impact viremia and neutralizing antibodies do not appear until months to years after the acute infection [13]. Beyond this, there is limited understanding of human immune responses to LASV infection, as civil conflict in Sierra Leone and Liberia and the subsequent decimation of the healthcare infrastructure in the region have effectively halted

Lassa fever research and, importantly, impeded the care of infected patients.

The report by McElroy and colleagues in this issue of *The Journal of Infectious Diseases* provides an alternative strategy to advance the science of Lassa fever and the care of infected patients. Capitalizing on the opportunity presented when a healthcare worker with acute Lassa fever was repatriated from West Africa to Emory University Hospital in Atlanta, McElroy and colleagues, in a collaboration with the Centers for Disease Control and Prevention, conducted an in-depth investigation to characterize the kinetics, magnitude, and quality of the innate and cellular immune response during primary LASV infection, while also providing advanced clinical care. Although they detail the immune response of a single patient, the comprehensive nature of their analysis offers a rare insight into the interaction of a high-consequence pathogen with a human host and an opportunity to identify critical aspects that require further investigation. After a tragically prolonged pause in Lassa fever research, the work reported by these investigators is an important step in moving the science of this neglected virus into the 21st century.

The detailed analysis of the kinetics of the immune response revealed a correlation between multiple components of the immune response and viral clearance including interferon alpha (IFN- α) expression, the appearance of LASV-specific immunoglobulin M (IgM), and increasing activated CD8⁺ T cells. The IFN- α findings support prior work in nonhuman primate models of Lassa fever linking IFN- α levels with survival as well as

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in vitro experiments of LASV-infected macrophages in which decreased viral replication in the presence of IFN- α and the persistence of LASV replication in IFNR^{-/-} mice [14, 15]. Although IgM levels correlated with viral clearance, the late appearance of neutralizing antibodies and a high neutralizing antibody requirement for protection in passive transfer experiments suggest that the IgM is unlikely to be protective. However, the authors offer alternative roles for the humoral response, including antibody-dependent complement fixation, which could be further evaluated in models of Lassa fever. Perhaps of most interest is that CD8⁺ T cells demonstrated biphasic activation: The first correlated with viral clearance, but the second occurring peak coincided with markers of proliferation and the expression of perforin and granzyme B and corresponded to the onset of diffuse lymphadenitis and epididymitis more than a month after symptom onset, in the absence of detectable viremia. Sustained activated CD8⁺ T cells during convalescence strongly suggests the persistence of LASV or viral antigen, further supported by the detection of LASV in the patient's semen beyond clearance of viremia. Collectively, this detailed immunologic study of a single patient highlights not only the potential role of IFN- α in viral clearance and the longitudinal immunophenotyping of CD8⁺ T cells during Lassa fever, but also identifies a finding of profound public health significance that requires further investigation—the seminal persistence of LASV.

Although LASV is found in virtually every body fluid and compartment during acute infection, little is known about the viral kinetics of LASV in body fluids other than blood. This case report provides further evidence that, despite the clearance of virus from the blood, some survivors continue to shed virus or viral RNA in immunologically protected compartments, including the urogenital system. Semen was positive by quantitative reverse-transcription polymerase chain reaction on days 15, 20 and 48, with evidence of virus isolation on day 20 [14]. In the absence

of a systematic survey or a validated test for urogenital fluids, it is not known for how long male survivors shed LASV in semen, or whether the virus can be found in female genital fluids. The implications of viral persistence in such immune sanctuaries are now just being recognized as a potential source of new outbreaks through sexual transmission for a number of other emerging infectious viruses, including Ebola and Zika viruses [15–17]. Therefore, although this case report tells the story of one man, the identification of viral persistence in semen draws critical attention to the need to follow survivors longitudinally after clearance of viremia and to apply validated assays to the study of LASV in body fluids other than blood.

Lassa fever is among the most important, but underrecognized, VHF public health threats. The recent EVD outbreak that quickly devastated parts of West Africa and triggered a public health crisis infected >28 000 individuals and killed >11 000. Because of the endemic nature of LASV, Lassa fever causes even more suffering, albeit at a slower and steadier pace. Fortunately, the repatriated healthcare worker described in this report survived. But many of those in the Lassa fever-endemic region who become infected do not survive, and there has been little reason for hope. The work presented here provides a blueprint for further research on the immune response and viral persistence during acute and convalescent Lassa fever and we hope that this work can be translated into improved clinical care.

In the wake of the most recent EVD outbreak, infrastructure for EVD surveillance, case management, and clinical research has been developed in West Africa, but still no such capability exists for Lassa fever, despite the predictable ongoing toll of this contagious disease. Vast sums were invested into the building of this infrastructure to respond to and research EVD in West Africa, and these resources should serve a dual purpose by allowing the growing clinical research community in the region to conduct more in-depth and collaborative studies of this

persistent threat. Laboratories stood up to provide Ebola virus diagnostics, and these could be turned toward developing validated and accessible assays for LASV detection. Infection control procedures implemented during the EVD outbreak could become routine in medical facilities where Lassa fever is endemic, so that healthcare workers can be protected. The processes that allowed for epidemiological data collection of EVD cases can also be applied to providing reasonable estimates of the toll and location of Lassa fever that can direct public health resources. Thirty years ago, an outbreak of horrific violence halted Lassa fever research. The recent outbreak of Ebola can and should resurrect this effort so that the people of West Africa, who suffer so greatly, can be free of its constant and deadly presence.

Notes

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