Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Antibody therapy for Lassa fever

Robert W Cross[1,2,7], Kathryn M Hastie[3,7], Chad E Mire[1,2], James E Robinson[4], Thomas W Geisbert[1,2], Luis M Branco[5], Erica Ollmann Saphire[3] and Robert F Garry[5,6]

Serum from convalescent Lassa fever patients was previously shown to be ineffective as a source of protective antibodies in some early studies. Subsequently, monoclonal antibodies (MAbs) to the Lassa virus (LASV) glycoprotein produced by memory B cells of West African patients who survived Lassa fever were identified. Development of MAbs as potential Lassa immunotherapeutics was facilitated by structural studies and mutational analyses that identified protective epitopes on the prefusion form of the LASV glycoprotein. Human mAbs were screened for reactivity to different neutralizing epitopes, potency, and broad reactivity against multiple lineages of LASV. MAbs were downselected in a guinea pig model of Lassa fever. A cocktail of three human MAbs designated Arevirumab-3 rescued 100% of *Cynomolgus macaques* at advanced stages of disease more than a week post-infection. Antibody therapeutics may be further developed in clinical trials in endemic areas potentially offering a key treatment option for Lassa fever.

Addresses
[1] Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, TX 77555, USA
[2] Galveston National Laboratory, University of Texas Medical Branch at Galveston, Galveston, TX 77555, USA
[3] La Jolla Institute for Immunology, La Jolla, CA 92037, USA
[4] Department of Pediatrics, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, USA
[5] Zalgen Labs, LLC, Germantown, MD 20876, USA
[6] Department of Microbiology and Immunology, Tulane University School of Medicine, 1430 Tulane Avenue, New Orleans, LA 70112, USA

Corresponding author: Garry, Robert F (rfgarry@tulane.edu)
[7] These authors contributed equally.

Current Opinion in Virology 2019, **37**:97–104

This review comes from a themed issue on **Lassa viruses**

Edited by **Connie Schmaljohn** and **David Safronetz**

For a complete overview see the Issue and the Editorial

Available online 8th August 2019

https://doi.org/10.1016/j.coviro.2019.07.003

Introduction

Lassa fever was first described in 1969 following the deaths of two missionary nurses during a hospital outbreak near Lassa, Nigeria [1••,2,3]. Lassa virus (LASV), a novel arenavirus was isolated from the blood of a surviving nurse [4•,5]. Two researchers were infected with LASV during these initial studies, one fatally [6]. The surviving scientist received blood donated from the surviving nurse as passive immunotherapy [6,7]. In the ensuing 50 years, there have been major advances in the understanding of LASV molecular biology, immunology and genomics [8••,9•,10••]. However, there are current no approved therapeutics or vaccines for treatment of Lassa fever. The populations of Sierra Leone, Guinea, Liberia, Nigeria, and other West African countries remain at high risk [11–16].

Humans acquire Lassa virus (LASV) infection primarily via exposure to virus-containing urine and feces of *Mastomys natalensis*, the natal mastomys or multimammate mouse. Additional rodent reservoirs/spillover hosts may also be involved in LASV transmission to humans [17]. The febrile illness induced by LASV begins 1–3 weeks after infection. After 4–7 days of mild illness, an estimated 20% of infected individuals develop symptoms of a severe viral hemorrhagic fever (VHF). Sore throat, vomiting and coughing are common [18–20]. Approximately 40% of Lassa fever patients experience bleeding from the nose, mouth, other orifices and mucosal surfaces. Death from Lassa fever generally occurs between 10–14 days of symptom onset and is attributed to diminished effective circulating volume, shock, and multi-organ system failure [21]. Infection is ~90% lethal to women in the third trimester of pregnancy. Unilateral or bilateral deafness is a common sequela. Case fatality rates (CFRs) in viremic patients range from approximately 25–30% in Nigeria [22,23] to 70% in Sierra Leone [24]. Lassa virus (LASV), the etiological agent of Lassa fever, is genetically divergent across the Lassa fever zone in West Africa, with at least six lineages [9•,25–27]. The contribution of viral genetic factors to the distinct CFRs in Nigeria and Sierra Leone is unknown.

Intensive supportive care, including fluid replacement and dialysis, appears to be beneficial in clinical management of Lassa fever patients [23]. Despite early anecdotal studies, including the aforementioned case [1••,28], the use of passive antibody for treatment of Lassa fever was not supported in an early human study [29•] and has not been since pursued. The current therapeutic modality for treatment of LASV infection is off-label use of intravenous ribavirin, a purine nucleoside-analogue. Some studies of ribavirin have shown efficacy in animal models of Lassa fever [30,31], but a more recent study found a delay in disease development no effect on mortality [32]. Further, ribavirin appears useful only in the early stages of

acute LASV infection [33], and its use has been associated with significant adverse events [34]. Several small molecules that possess antiviral properties against arenaviruses are under evaluation for treatment of Lassa fever, including compounds originally identified by Siga Technologies [32,35]. Favipiravir has be shown to be effective at treating Lassa fever in both guinea pigs [36] and macaques [37]. Favipiravir was recently administered in combination with ribavirin to treat Lassa fever in two human patients in Frankfurt, Germany, and Atlanta, USA, who both survived [38]. Potently neutralizing monoclonal antibodies have been developed against different epitopes on the viral glycoprotein. Here, we discuss the potential use of LASV human monoclonal antibodies (huMAbs) as immunotherapeutics.

Antibodies for the treatment of Lassa fever

Neutralizing antibodies that physically inactivate or neutralize viral entry are important for control of several viral infections [39,40]. LASV expresses one surface protein, a glycoprotein that self-assembles into a complex termed the glycoprotein complex (GPC). GPC mediates LASV entry into target cells and is the primary target for antiviral antibodies [10••,41]. The virion form of GPC is a trimer of heterodimers. Each heterodimer contains a receptor-binding subunit GP1 and a transmembrane fusion subunit GP2 [42]. LASV also encodes an unusual stable signal peptide (SSP) that is required for proper processing of GPC and is retained in the virion as part of the GPC [43]. GPC, however, is metastable, conformationally labile, and heavily glycosylated. The thick carbohydrate coat and structural instability challenge the elicitation of neutralizing antibodies. Approximately half of humans who survive LASV infection fail to produce neutralizing antibodies, even late in convalescence [20,44].

The delayed development of neutralizing antibodies is reflected in the persistence of IgM antibodies to LASV GPC and perturbation of expected class switching to IgG in the course of human infection [45]. Perturbations of antibody class-switching were also observed in a non-human primate (NHP) model of LASV infection [46]. Neutralizing antibody may not be a factor in survival of humans acutely infected by LASV. However, the question of whether neutralizing antibodies with appropriate virus strain specificity and delivered in sufficient quantity can be an effective treatment for Lassa fever if provided passively has not been adequately tested. Further, it remains to be determined if protective neutralizing antibody could be elicited by vaccination, perhaps with engineered forms of GPC.

Early studies of passive antibody transfer evaluated only whole plasma which may have had low neutralizing titers or not matched the infecting strain. Some studies have met with success: plasma from human survivors of Lassa fever has been used to successfully treat Lassa fever in *Cynomolgus macaques* [47,48]. Further, several Lassa fever patients treated with survivor plasma survived [28]. Others have found that convalescent plasma was less effective than ribavirin in treating acute Lassa fever [29•]. In particular, the use of either sera with low titer of LASV-specific antibodies or sera containing antibodies not closely matching the infecting strain of LASV likely contributed to the relative ineffectiveness of passive antibody therapy in those studies [49].

In contrast, convalescent plasma is the only approved treatment for Argentine hemorrhagic fever caused by Junin virus (JUNV), a New World arenavirus [50,51]. For Argentine hemorrhagic fever, there is a direct relationship between delivery via passive transfer of an adequate dose of neutralizing antibodies and patient outcome. In a double-blind trial, patients with Argentine hemorrhagic fever treated with immune plasma within eight days of the onset of the disease had a much lower mortality rate than those given normal plasma [52•]. Further, generation of neutralizing antibodies has been established as a key measure of successful vaccination against JUNV [53].

Monoclonal antibodies (MAbs) have emerged as therapeutics for numerous diseases because of their low toxicity and high potency [54–57]. Synagis, a monoclonal antibody immunotherapeutic for human respiratory syncytial virus infection, has been approved for use for over 20 years, but remains the only FDA approved viral immunotherapeutic [58]. ZMapp, a cocktail of chimerized murine antibodies, along with other REGN-EB3 and mAb 114 are under evaluation during an Ebola outbreak in a conflict zone in the Democratic Republic of the Congo [59]. Immunotherapeutics are also under development for treating HIV and other viral diseases [60–63].

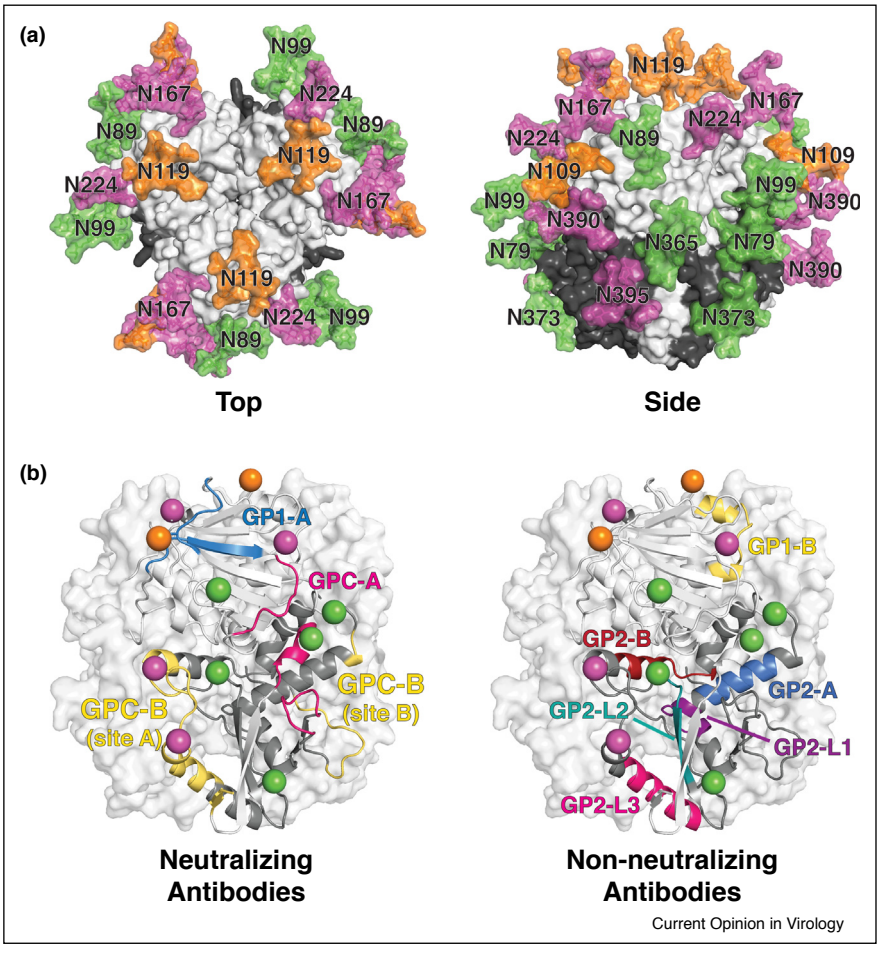
The need for treatments against Lassa fever, the potential for therapeutic use of mAbs, and opportunities to select mAbs for increased potency and broad reactivity against multiple lineages of LASV prompted us to isolate and characterize huMAbs from West African survivors. 113 mAbs were identified in patients who survived Lassa fever and developed sustained antibody titers to LASV antigens months or years into convalescence [10••]. One-half of the resulting panel of huMAbs bind the GP2 fusion subunit, one-fourth recognize the GP1 receptor-binding subunit, and the remaining fourth are specific for the assembled glycoprotein complex, requiring both GP1 and GP2 subunits for recognition. Most neutralizing antibodies require both the GP1 and GP2 subunits for binding and bind only to prefusion GPC. The majority of these neutralizing antibodies cluster into

a single competition group, termed GPC-B. Two huMAbs are contained in a distinct group termed GPC-A. Only a few bind to the GP1 subunit alone, in a competition group termed GP1-A. Lastly, a single antibody, 8.9F, recognizes only transmembrane GPC. 8.9F has an unusually long CDR3 of 33 amino acids and was derived from a person with likely multiple exposures to LASV. Compared with non-neutralizing mAbs, the neutralizing mAbs in the panel have higher binding affinities and greater divergence from germline progenitors. A subpanel of huMAbs neutralized all four LASV lineages that co-circulate in West Africa, including diverse lineages from Nigeria, and were classified as broadly neutralizing human monoclonal antibodies (BNhuMAbs).

The antigenic landscape of the prefusion LASV GP trimer

LASV GPC is heavily cloaked by substantial host-derived glycosylation making it a challenging target for therapeutics. LASV GPC has eleven N-linked glycosylation sites on each monomer, which together comprise ~30% of the total mass of the protein [8••,64] (Figure 1a). This extensive glycan shield leaves few regions vulnerable to antibody binding. Recent structural analysis of the prefusion trimer of LASV GP in complex with members of the GPC-B competition group demonstrated this major class of neutralizing antibodies recognizes a conserved and highly quaternary epitope at the base of the GPC trimer that spans the GP1 and GP2 subunits within a single monomer, and also bridges two neighboring monomers

Figure 1



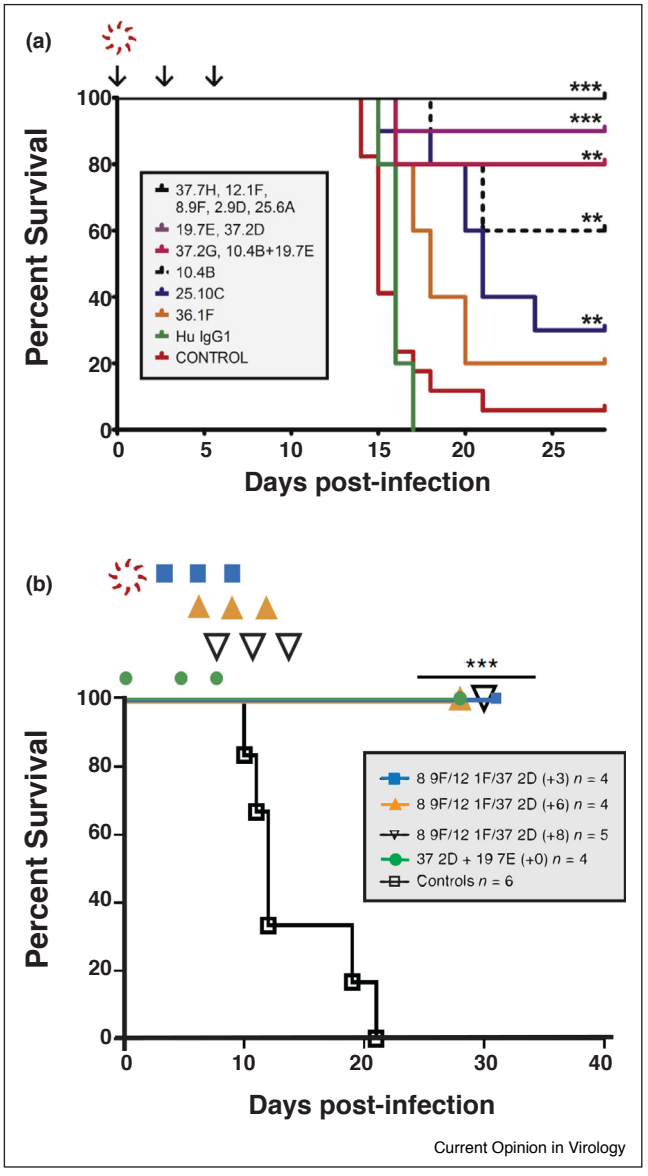
The antigenic landscape of GP.
(a) Modeling of the experimentally observed glycosylation [70] onto the structure of the LASV GP trimer (PDB 5VK2, [8••]). The GP1 subunit of each GP monomer is colored light gray and the GP2 subunit is colored dark gray. Glycans are colored according to the oligomannose content: 0–30% – magenta; 30–79% – orange; 80–100% – green. **(b)** Left: Neutralizing LASV antibodies, colored by epitope group: GP1-A, which recognizes the GP1 subunit alone is colored blue; GPC-A, which recognizes a single prefusion GP monomer is colored pink; GPC-B, which recognizes a quaternary epitope that spans two GP monomers (site a and b) within the trimer is colored yellow. Right: Non-neutralizing LASV antibodies, colored by epitope group. Conformational epitopes include: GP1-B (yellow); GP2-A (blue); GP2-B (red). Linear epitopes include: GP2-L1 (purple); GP2-L2 (cyan); GP2-L3 (pink). For both panels, glycosylated asparginine (Asn) residues are shown as spheres and are colored according to the scheme in (a).

together (Figure 1b). Structural analysis (Hastie *et al.*, submitted) coupled with escape mutations, deletion and mutagenesis studies [10••] has now localized the alternate neutralizing site, the GPC-A epitope, to a distinct expanse across GP1 and GP2 subunits and the GP1-A epitope to the β-sheet/apical domain of GP1 (Figure 1b). While GPC-B antibodies bridge neighboring monomers together, the GPC-A and GP1-A neutralizing antibodies bind one single GP monomer at a time. In contrast, epitopes of non-neutralizing antibodies map elsewhere, primarily to the interior, proteinaceous faces of LASV at the internal interfaces of the trimer. These non-neutralizing antibodies may have been elicited by non-functional, opened trimers as the proteinaceous inner surfaces become exposed. The neutralizing antibodies, such as those in the GPC-B, GPC-A, GP1-A, and 8.9F competition groups bind the outer surfaces of the GPC trimer, amidst the glycans that cloak its surface. These neutralizing antibodies must accommodate the glycan shield of LASV in molecular recognition.

Efficacy of neutralizing antibodies in nonhuman primate models

Neutralizing antibodies were tested in a uniformly lethal guinea pig model challenged with LASV Josiah, which educated downselection of huMAbs for testing in a NHP model of Lassa fever [65••] (Figure 2a). A cocktail of three BNhuMAbs was subsequently evaluated in walkout (delay-to-treatment) studies in NHP, resulting in complete protection, even when first treatment was administered as late as 8 days post challenge with LASV Josiah (lineage IV) [66••] (Figure 2b). Complete protection with the cocktail has also been observed against a newly isolated and characterized Nigeria lineage II LASV from Nigeria, which appears to cause a more aggressive diseases than the Josiah strain (Cross and Geisbert, unpublished). Importantly, complete clearance of circulating viremia was observed after a single treatment with the immunotherapeutic. The three-BNhuMAb cocktail (37.2D, 8.9F, 12.1F) under evaluation has been named Arevirumab-3. Recent studies demonstrated similar efficacy in NHP treated with two BNhuMAb combinations (Cross and Geisbert, unpublished). Complete protection was achieved with a specific two BNhuMAb cocktail in a dose down delay-to-treatment study with 1.5 mg/kg of each MAb, albeit with prolonged clinical illness and reduced clearance of viremia (Cross and Geisbert, unpublished). To date, Arevirumab-3 has demonstrated high efficacy in resolving advanced Lassa fever infections in NHP, is safe and well tolerated, and has not resulted in the emergence of escape mutant Lassa viruses *in vivo*. *In vitro* studies have demonstrated that Arevirumab-3 completely neutralizes the virus in culture and does not give rise to escape mutant viruses, thus supporting further development of this cocktail as a potential future treatment for Lassa virus infections. Gradual reduction in the production cost of MAbs for injection, coupled with the low doses required to clear LASV infection in NHP, further warrant consideration of immunotherapeutics as a novel, frontline therapeutic option for Lassa fever and viral hemorrhagic fevers in general.

Figure 2



Protective efficacy profile of LASV GPC huMAbs in a Hartley guinea pig and *Cynomolgus macaque* models of Lassa fever.
(a) Pooled survival plots for treated and control guinea pigs challenged with guinea pig-adapted LASV strain Josiah LASV from multiple independent experiments, as noted. Control guinea pigs succumbed to Lassa fever between 14 and 22 PI (median 15 days). A single guinea pig survived infection through the 28-day study endpoint (1/18). **(b)** Protective-efficacy profile of LASV huMAb combination therapy in a *Cynomolgus macaque* model of Lassa fever. Kaplan–Meier survival curve for each cohort treated with a cocktail of anti-LASV huMAbs after LASV challenge. (37.2D + 19.7E) or day 3 (+3), day 6 (+6), or day 8 (+8) post LASV challenge. ****$P < 0.0001$; as determined by Mantel–Cox test.

Discussion

The ineffectiveness, in some early studies, of serum from convalescent Lassa fever patients as a source of protective antibodies delayed efforts to develop immunotherapeutics for post-exposure treatment. Recent encouraging results using well-characterized BNhuMAbs in animal models, however, are now driving the development of potent immunotherapeutic cocktails for the treatment and prevention of Lassa fever [65••,66••]. Studies of Lassa GPC structure and interaction with antibodies provide a foundation for understanding the molecular mechanisms of antibody biogenesis, specificity and protection against LASV infection in humans, and eventual clinical treatment of acute Lassa fever.

LASV GPC is a metastable, conformationally labile, heavily glycosylated molecules that more frequently elicits non-neutralizing antibodies than neutralizing antibodies. Such non-neutralizing antibodies may promote Fc-mediated signaling to destroy viruses and infected cells [10••], and indeed recent studies document the potential role of non-neutralizing antibodies in protection against fatal Lassa fever. Notably, a LASV vaccine candidate, based on a recombinant rabies virus expressing wild-type LASV GPC, was reported to induce protection in rodent models of Lassa fever, even though it failed to induce detectable levels of neutralizing antibodies [67]. Protection was instead linked to the appearance and titer of non-neutralizing, Fc-mediated cell-targeting antibodies. Further studies to define the role of non-neutralizing epitopes in survival of LASV infection are necessary to develop optimal immunotherapeutics and vaccines. Neutralizing antibodies, which may be elicited against well-folded trimeric GPC, and Fc-mediated cell killing antibodies, which may be elicited against any conformation of GP could provide complementary activities in control of infection. Indeed, a combination of both neutralizing and Fc-mediated cell killing activities would be ideal in post-exposure therapeutics and as elicited by vaccines alike.

The World Health Organization (WHO) and the Coalition for Epidemic Preparedness Innovations (CEPI) have each designated LASV as a priority pathogen [68,69]. These rankings are based on the potential for further geographic expansion of the rodent reservoirs, the ease of procurement and weaponization of the virus, the frequent importation of LASV to North America and Europe, and the emergence of novel strains in densely populated West Africa. The next decade could produce important advances in control and treatment of Lassa fever. Novel therapeutics such as Arevirumab should progress toward advanced testing. Challenges of immunotherapeutics relate to the costs of production and deployment to under resourced West Africa. Cost-effective processes for production of MAbs and emphasis on highly potent MAbs will be required. Regulatory approval will also remain a challenge, but human clinical trials in endemic regions should be performed to test safety and efficacy. After well-controlled clinical trials, antibody therapeutics may be developed and offer a key treatment option for an endemic disease that otherwise claims thousands of lives each year and leaves numerous survivors with profound deafness and other sequelae.

Funding

This work was supported by contract HSN272200900049C from the National Institutes of Health (NIH) and by NIH grants AI067188, AI067927, AI070530, AI081982, AI082119, AI082805AI088843, AI104216, AI104621, AI115754, P20GM103501, and 1U01HG007480-01, and grants from the World Bank, the Bill and Melinda Gates Foundation and the Paul G. Allen Family Foundation.

Conflict of interest statement

The Viral Hemorrhagic Fever Consortium (http://www.vhfc.org) is a public–private partnership of academic and industry scientists who are developing diagnostic tests, therapeutic agents (including immunotherapeutics) and vaccines for Lassa fever, Ebola and other severe diseases. Tulane University and its various academic and industry partners have filed United States and foreign patent applications on behalf of the VHFC for several technologies that have resulted from these efforts. Technical information may also be kept as trade secrets. If commercial products are developed, consortium members may receive royalties or profits. This does not alter our adherence to all policies of Current Opinion in Virology, NIH and other funding agencies on sharing data and materials.

Acknowledgements

Deborah H. Elliott, Julie A. Rouelle, Chandrika B. Kannadka, Ashley A. Smira, Courtney E. Garry, Benjamin T. Bradley, Haini Yu, and Rachael E. Yenni isolated and characterized the huMabs discussed in this review. Michelle A. Zandonatti contributed to the structural characterization of the LASV GPC trimer. Joan B. Geisbert, Viktoriya Borisevich, Krystle N. Agans, Daniel J. Deer, and Karla A. Fenton performed immunotherapeutic studies in guinea pigs and monkeys at BSL-4. Megan L. Heinrich, Megan M. Rowland, and Anatoliy P. Koval purified huMAbs for these studies. Mathew L. Boisen, Diana K. S. Nelson, and Duane J. Bush developed LASV immunoassays used to identify and characterize LASV huMAbs. Augustine Goba, Mambu Momoh, Mohamed Fullah, and John Demby Sandi characterized immune responses to LASV in Sierra Leonean patients. Donald S. Grant, S. Humarr Khan, Michael Gbakie, Mbalu Fonnie, Veronica J. Koroma, and Lansana Kanneh provided clinical care for Lassa fever patients in Sierra Leone and obtained blood for huMAb isolation from Lassa fever survivors. Christian T. Happi, Odia Ikponmwosa, Philomema Emeron, Danny A. Asogun, Peter O. Okokhere, and Onikepe O. Folarin provided clinical care for Lassa fever patients in Nigeria and obtained blood for huMAb isolation from Lassa fever survivors. John S. Schieffelin is clinical director of the Viral Hemorrhagic Fever Consortium (VHFC) and Jeffrey G. Shaffer is data manager of the VHFC; this work could not proceed without their efforts. Kayla G. Barnes, Anna E. Lachenauer, Aaron E. Lin, Mahan Nekoui, Dylan Kotliar, Sarah M. Winnicki, Danny Park, Nathan Yozwiak, Katherine J. Siddle, Matthias Pauthner, Christian T Happi, Kristian G. Andersen, and Pardis C. Sabeti defined LASV genetic diversity. Saori Sakabe, Brian Sullivan, Juan Carlos de la Torre, Stephanie Lavergne, and Michael B.A. Oldstone remind us that cellular immune responses to LASV are important for recovery from natural infection and vaccine response. Christopher M. Bishop, Tynette Hills, Lilia Melnik, Brandon Beddingfield, Andrew Hoffman, Allison Smither, Antoinette Bell, Simbirie Jalloh, and Allyson Haislip provide logistical and scientific support

and program management. We also recognize the support of the Sierra Leonean Ministry of Health and Sanitation, the Nigerian Ministry of Health and other members of the VHFC not named here. We are also grateful for the contributions of Lassa fever patients, their families and communities.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Frame JD, Baldwin JM Jr, Gocke DJ, Troup JM: **Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings**. *Am J Trop Med Hyg* 1970, **19**:670-676.

First characterization of Lassa fever in three missionary nurses working in northeastern Nigeria.

2. Troup JM, White HA, Fom AL, Carey DE: **An outbreak of Lassa fever on the Jos plateau, Nigeria, in January-February 1970. A preliminary report**. *Am J Trop Med Hyg* 1970, **19**:695-696.

3. Carey DE, Kemp GE, White HA, Pinneo L, Addy RF, Fom AL, Stroh G, Casals J, Henderson BE: **Lassa fever. Epidemiological aspects of the 1970 epidemic, Jos, Nigeria**. *Trans R Soc Trop Med Hyg* 1972, **66**:402-408.

4. • Buckley SM, Casals J: **Lassa fever, a new virus disease of man from West Africa. 3. Isolation and characterization of the virus**. *Am J Trop Med Hyg* 1970, **19**:680-691.

Initial characterization of LASV.

5. Buckley SM, Casals J, Downs WG: **Isolation and antigenic characterization of Lassa virus**. *Nature* 1970, **227**:174.

6. Watts GM: **Lily Lyman Pinneo**. *Lancet* 2013, **380**:1552.

7. Bond N, Schieffelin JS, Moses LM, Bennett AJ, Bausch DG: **A historical look at the first reported cases of Lassa fever: IgG Antibodies 40 years after acute infection**. *Am J Trop Med Hyg* 2012, **88**:241-244.

8. •• Hastie KM, Zandonatti MA, Kleinfelter LM, Heinrich ML, Rowland MM, Chandran K, Branco LM, Robinson JE, Garry RF, Saphire EO: **Structural basis for antibody-mediated neutralization of Lassa virus**. *Science* 2017, **356**:923-928.

First characterization of the prefusion (virion) form of the glycoprotein of any arenavirus. The structure was determined after binding a GPC-B huMAb.

9. • Andersen KG, Shapiro BJ, Matranga CB, Sealfon R, Lin AE, Moses LM, Folarin OA, Goba A, Odia I, Ehiane PE *et al.*: **Clinical sequencing uncovers origins and evolution of Lassa virus**. *Cell* 2015, **162**:738-750.

Genomic study showing the potential movement of LASV across West Africa.

10. •• Robinson JE, Hastie KM, Cross RW, Yenni RE, Elliott DH, Rouelle JA, Kannadka CB, Smira AA, Garry CE, Bradley BT *et al.*: **Most neutralizing human monoclonal antibodies target novel epitopes requiring both Lassa virus glycoprotein subunits**. *Nat Commun* 2016, **7**:11544.

This study characterizes the first panel of human antibodies against Lassa virus and determines that most neutralizing antibodies against Lassa virus recognize epitopes displayed only on the prefusion glycoprotein trimer.

11. Keane E, Gilles HM: **Lassa fever in Panguma Hospital, Sierra Leone, 1973-6**. *Br Med J* 1977, **1**:1399-1402.

12. Bausch DG, Demby AH, Coulibaly M, Kanu J, Goba A, Bah A, Conde N, Wurtzel HL, Cavallaro KF, Lloyd E *et al.*: **Lassa fever in Guinea: I. Epidemiology of human disease and clinical observations**. *Vector Borne Zoonotic Dis* 2001, **1**:269-281.

13. Fraser DW, Campbell CC, Monath TP, Goff PA, Gregg MB: **Lassa fever in the Eastern Province of Sierra Leone, 1970-1972. I. Epidemiologic studies**. *Am J Trop Med Hyg* 1974, **23**:1131-1139.

14. Mertens PE, Patton R, Baum JJ, Monath TP: **Clinical presentation of Lassa fever cases during the hospital epidemic at Zorzor, Liberia, March-April 1972**. *Am J Trop Med Hyg* 1973, **22**:780-784.

15. Monath TP, Mertens PE, Patton R, Moser CR, Baum JJ, Pinneo L, Gary GW, Kissling RE: **A hospital epidemic of Lassa fever in Zorzor, Liberia, March-April 1972**. *Am J Trop Med Hyg* 1973, **22**:773-779.

16. Safronetz D, Lopez JE, Sogoba N, Traore SF, Raffel SJ, Fischer ER, Ebihara H, Branco L, Garry RF, Schwan TG *et al.*: **Detection of Lassa virus, Mali**. *Emerg Infect Dis* 2010, **16**:1123-1126.

17. **Characterization of contacts of the population of Guinea with synanthropic rodents as Lassa fever virus carriers**. *Med Parazitol (Mosk)* 2007:47-51.

18. Knobloch J, McCormick JB, Webb PA, Dietrich M, Schumacher HH, Dennis E: **Clinical observations in 42 patients with Lassa fever**. *Tropenmed Parasitol* 1980, **31**:389-398.

19. Richmond JK, Baglole DJ: **Lassa fever: epidemiology, clinical features, and social consequences**. *BMJ* 2003, **327**:1271-1275.

20. McCormick JB, Fisher-Hoch SP: **Lassa fever**. *Curr Top Microbiol Immunol* 2002, **262**:75-109.

21. Peters CJ, Liu CT, Anderson GW Jr, Morrill JC, Jahrling PB: **Pathogenesis of viral hemorrhagic fevers: Rift Valley fever and Lassa fever contrasted**. *Rev Infect Dis* 1989, **11(Suppl. 4)**:S743-749.

22. Ilori EA, Frank C, Dan-Nwafor CC, Ipadeola O, Krings A, Ukponu W, Womi-Eteng OE, Adeyemo A, Mutbam SK, Musa EO *et al.*: **Increase in Lassa fever cases in Nigeria, January-March 2018**. *Emerg Infect Dis* 2019, **25**.

23. Okokhere P, Colubri A, Azubike C, Iruolagbe C, Osazuwa O, Tabrizi S, Chin E, Asad S, Ediale E, Rafiu M *et al.*: **Clinical and laboratory predictors of Lassa fever outcome in a dedicated treatment facility in Nigeria: a retrospective, observational cohort study**. *Lancet Infect Dis* 2018, **18**:684-695.

24. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M *et al.*: **Lassa fever in post-conflict Sierra Leone**. *PLoS Negl Trop Dis* 2014, **8**: e2748.

25. Siddle KJ, Eromon P, Barnes KG, Mehta S, Oguzie JU, Odia I, Schaffner SF, Winnicki SM, Shah RR, Qu J *et al.*: **Genomic analysis of Lassa virus during an increase in cases in Nigeria in 2018**. *NEJM* 2018, **379**:1745-1753.

26. Safronetz D, Strong JE, Feldmann F, Haddock E, Sogoba N, Brining D, Geisbert TW, Scott DP, Feldmann H: **A recently isolated Lassa virus from Mali demonstrates atypical clinical disease manifestations and decreased virulence in *Cynomolgus macaques***. *J Infect Dis* 2013, **207**:1316-1327.

27. Whitmer SLM, Strecker T, Cadar D, Dienes HP, Faber K, Patel K, Brown SM, Davis WG, Klena JD, Rollin PE *et al.*: **New lineage of Lassa Virus, Togo, 2016**. *Emerg Infect Dis* 2018, **24**:599-602.

28. Frame JD, Verbrugge GP, Gill RG, Pinneo L: **The use of Lassa fever convalescent plasma in Nigeria**. *Trans R Soc Trop Med Hyg* 1984, **78**:319-324.

29. • McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliott LH, Belmont-Williams R: **Lassa fever. Effective therapy with ribavirin**. *N Engl J Med* 1986, **314**:20-26.

Demonstration that ribavirin treatment was more effective than convalescent plasma in treating Lassa fever.

30. Stephen EL, Jahrling PB: **Experimental Lassa fever virus infection successfully treated with ribavirin**. *Lancet* 1979, **1**:268-269.

31. Wannarka GL, Stephen EL, Canonico PG: **Preclinical evaluation of monkeys of a ribavirin regimen proposed for use in Lassa fever patients**. *Toxicol Appl Pharmacol* 1982, **64**:155-159.

32. Cashman KA, Smith MA, Twenhafel NA, Larson RA, Jones KF, Allen RD 3rd, Dai D, Chinsangaram J, Bolken TC, Hruby DE *et al.*: **Evaluation of Lassa antiviral compound ST-193 in a guinea pig model**. *Antiviral Res* 2011, **90**:70-79.

33. Hadi CM, Goba A, Khan SH, Bangura J, Sankoh M, Koroma S, Juana B, Bah A, Coulibaly M, Bausch DG: **Ribavirin for Lassa fever postexposure prophylaxis**. *Emerg Infect Dis* 2010, **16**:2009-2011.

34. Fisher-Hoch SP, Gborie S, Parker L, Huggins J: **Unexpected adverse reactions during a clinical trial in rural west Africa**. *Antiviral Res* 1992, **19**:139-147.

35. Madu IG, Files M, Gharaibeh DN, Moore AL, Jung KH, Gowen BB, Dai D, Jones KF, Tyavanagimatt SR, Burgeson JR *et al.*: **A potent Lassa virus antiviral targets an arenavirus virulence determinant**. *PLoS Pathog* 2018, **14**:e1007439.

36. Safronetz D, Rosenke K, Westover JB, Martellaro C, Okumura A, Furuta Y, Geisbert J, Saturday G, Komeno T, Geisbert TW *et al.*: **The broad-spectrum antiviral favipiravir protects guinea pigs from lethal Lassa virus infection post-disease onset**. *Sci Rep* 2015, **5**:14775.

37. Rosenke K, Feldmann H, Westover JB, Hanley PW, Martellaro C, Feldmann F, Saturday G, Lovaglio J, Scott DP, Furuta Y *et al.*: **Use of Favipiravir to treat Lassa virus infection in Macaques**. *Emerg Infect Dis* 2018, **24**:1696-1699.

38. Raabe VN, Kann G, Ribner BS, Morales A, Varkey JB, Mehta AK, Lyon GM, Vanairsdale S, Faber K, Becker S *et al.*: **Favipiravir and Ribavirin treatment of epidemiologically linked cases of Lassa fever**. *Clin Infect Dis* 2017, **65**:855-859.

39. Christensen SR, Toulmin SA, Griesman T, Lamerato LE, Petrie JG, Martin ET, Monto AS, Hensley SE: **Assessing the protective potential of H1N1 influenza virus hemagglutinin head and stalk antibodies in humans**. *J Virol* 2019, **93** 2134-18.

40. Hasan SS, Sun C, Kim AS, Watanabe Y, Chen CL, Klose T, Buda G, Crispin M, Diamond MS, Klimstra WB *et al.*: **Cryo-EM structures of eastern equine encephalitis virus reveal mechanisms of virus disassembly and antibody neutralization**. *Cell Rep* 2018, **25**:3136-3147 e3135.

41. Sommerstein R, Flatz L, Remy MM, Malinge P, Magistrelli G, Fischer N, Sahin M, Bergthaler A, Igonet S, Ter Meulen J *et al.*: **Arenavirus glycan shield promotes neutralizing antibody evasion and protracted infection**. *PLoS Pathog* 2015, **11**: e1005276.

42. Li S, Sun Z, Pryce R, Parsy ML, Fehling SK, Schlie K, Siebert CA, Garten W, Bowden TA, Strecker T *et al.*: **Acidic pH-induced conformations and LAMP1 binding of the lassa virus glycoprotein spike**. *PLoS Pathog* 2016, **12**:e1005418.

43. Nunberg JH, York J: **The curious case of arenavirus entry, and its inhibition**. *Viruses* 2012, **4**:83-101.

44. Fisher-Hoch SP, McCormick JB, Auperin D, Brown BG, Castor M, Perez G, Ruo S, Conaty A, Brammer L, Bauer S: **Protection of rhesus monkeys from fatal Lassa fever by vaccination with a recombinant vaccinia virus containing the Lassa virus glycoprotein gene**. *Proc Natl Acad Sci U S A* 1989, **86**:317-321.

45. Branco LM, Grove JN, Boisen ML, Shaffer JG, Goba A, Fullah M, Momoh M, Grant DS, Garry RF: **Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection**. *Virol J* 2011, **8**:478.

46. Niklasson BS, Jahrling PB, Peters CJ: **Detection of Lassa virus antigens and Lassa virus-specific immunoglobulins G and M by enzyme-linked immunosorbent assay**. *J Clin Microbiol* 1984, **20**:239-244.

47. Jahrling PB, Peters CJ: **Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain**. *Infect Immun* 1984, **44**:528-533.

48. Jahrling PB, Peters CJ, Stephen EL: **Enhanced treatment of Lassa fever by immune plasma combined with ribavirin in cynomolgus monkeys**. *J Infect Dis* 1984, **149**:420-427.

49. Jahrling PB, Frame JD, Rhoderick JB, Monson MH: **Endemic Lassa fever in Liberia. IV. Selection of optimally effective plasma for treatment by passive immunization**. *Trans R Soc Trop Med Hyg* 1985, **79**:380-384.

50. Enria DA, Briggiler AM, Sanchez Z: **Treatment of Argentine hemorrhagic fever**. *Antiviral Res* 2008, **78**:132-139.

51. Enria D, Franco SG, Ambrosio A, Vallejos D, Levis S, Maiztegui J: **Current status of the treatment of Argentine hemorrhagic fever**. *Med Microbiol Immunol* 1986, **175**:173-176.

52. • Enria DA, Briggiler AM, Fernandez NJ, Levis SC, Maiztegui JI: **Importance of dose of neutralising antibodies in treatment of Argentine haemorrhagic fever with immune plasma**. *Lancet* 1984, **2**:255-256.

Defines role of neutralizing antibody in immunotherapy of Argentine hemorrhagic fever.

53. Maiztegui JI, McKee KT Jr, Barrera Oro JG, Harrison LH, Gibbs PH, Feuillade MR, Enria DA, Briggiler AM, Levis SC, Ambrosio AM *et al.*: **Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group**. *J Infect Dis* 1998, **177**:277-283.

54. Julg B, Liu PT, Wagh K, Fischer WM, Abbink P, Mercado NB, Whitney JB, Nkolola JP, McMahan K, Tartaglia LJ *et al.*: **Protection against a mixed SHIV challenge by a broadly neutralizing antibody cocktail**. *Sci Transl Med* 2017, **9**.

55. Marston HD, Paules CI, Fauci AS: **Monoclonal antibodies for emerging infectious diseases – borrowing from history**. *N Engl J Med* 2018, **378**:1469-1472.

56. Kisalu NK, Idris AH, Weidle C, Flores-Garcia Y, Flynn BJ, Sack BK, Murphy S, Scho NA, Freire E, Francica JR *et al.*: **A human monoclonal antibody prevents malaria infection by targeting a new site of vulnerability on the parasite**. *Nat Med* 2019, **25**:188-189.

57. Gaudinski MR, Coates EE, Houser KV, Chen GL, Yamshchikov G, Saunders JG, Holman LA, Gordon I, Plummer S, Hendel CS *et al.*: **Safety and pharmacokinetics of the Fc-modified HIV-1 human monoclonal antibody VRC01LS: a Phase 1 open-label clinical trial in healthy adults**. *PLoS Med* 2018, **15**:e1002493.

58. DeVincenzo JP, Hall CB, Kimberlin DW, Sanchez PJ, Rodriguez WJ, Jantausch BA, Corey L, Kahn JS, Englund JA, Suzich JA *et al.*: **Surveillance of clinical isolates of respiratory syncytial virus for palivizumab (Synagis)-resistant mutants**. *J Infect Dis* 2004, **190**:975-978.

59. Friedrich MJ: **Multidrug Ebola trial underway in democratic republic of Congo**. *JAMA* 2019, **321**:637.

60. Allen ER, Krumm SA, Raghwani J, Halldorsson S, Elliott A, Graham VA, Koudriakova E, Harlos K, Wright D, Warimwe GM *et al.*: **A protective monoclonal antibody targets a site of vulnerability on the surface of rift valley Fever virus**. *Cell Rep* 2018, **25**:3750-3758 e3754.

61. Li J, Watterson D, Chang CW, Che XY, Li XQ, Ericsson DJ, Qiu LW, Cai JP, Chen J, Fry SR *et al.*: **Structural and functional characterization of a cross-reactive dengue virus neutralizing antibody that recognizes a cryptic epitope**. *Structure* 2018, **26**:51-59.e54.

62. Milligan JC, Parekh DV, Fuller KM, Igarashi M, Takada A, Saphire EO: **Structural characterization of Pan-ebolavirus antibody 6D6 Targeting the fusion peptide of the surface glycoprotein**. *J Infect Dis* 2019, **219**:415-419.

63. Mendoza P, Gruell H, Nogueira L, Pai JA, Butler AL, Millard K, Lehmann C, Suarez I, Oliveira TY, Lorenzi JCC *et al.*: **Combination therapy with anti-HIV-1 antibodies maintains viral suppression**. *Nature* 2018, **561**:479-484.

64. Eichler R, Lenz O, Garten W, Strecker T: **The role of single N-glycans in proteolytic processing and cell surface transport of the Lassa virus glycoprotein GP-C**. *Virol J* 2006, **3**:41.

65. •• Cross RW, Mire CE, Branco LM, Geisbert JB, Rowland MM, Heinrich ML, Goba A, Momoh M, Grant DS, Fullah M *et al.*: **Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies**. *Antiviral Res* 2016, **133**:218-222.

Downselection of huMAbs for protective efficacy in a guinea pig model of Lassa fever.

66. •• Mire CE, Cross RW, Geisbert JB, Borisevich V, Agans KN, Deer DJ, Heinrich ML, Rowland MM, Goba A, Momoh M *et al.*: **Human-monoclonal-antibody therapy protects nonhuman primates against advanced Lassa fever**. *Nat Med* 2017, **23**:1146-1149.

Rescue of monkeys from fatal Lassa fever with Arevirumab-3.

67. Abreu-Mota T, Hagen KR, Cooper K, Jahrling PB, Tan G, Wirblich C, Johnson RF, Schnell MJ: **Non-neutralizing antibodies elicited by recombinant Lassa-Rabies vaccine are critical for protection against Lassa fever**. *Nat Commun* 2018, **9**:4223.

68. World Health Organization: *List of Blueprint Priority Diseases*. 2018 https://www.who.int/blueprint/priority-diseases/en/.

69. Burki T: **CEPI: preparing for the worst**. *Lancet Infect Dis* 2017, **17**:265-266.

70. Watanabe Y, Raghwani J, Allen JD, Seabright GE, Li S, Moser F, Huiskonen JT, Strecker T, Bowden TA, Crispin M: **Structure of the Lassa virus glycan shield provides a model for immunological resistance**. *Proc Natl Acad Sci U S A* 2018, **115**:7320-7325.