Treatment of Lassa virus infection in outbred guinea pigs with first-in-class human monoclonal antibodies

Robert W. Cross a, b, 1, Chad E. Mire a, b, 1, Luis M. Branco c, 1, Joan B. Geisbert a, b, Megan M. Rowland c, Megan L. Heinrich c, Augustine Goba d, e, Mambu Momoh d, e, f, Donald S. Grant d, e, Mohamed Fullah d, e, 2, Sheik Humarr Khan d, e, 2, James E. Robinson g, Thomas W. Geisbert a, b, *, Robert F. Garry c, h

a Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, TX, USA
b Galveston National Laboratory, University of Texas Medical Branch at Galveston, Galveston, TX, USA
c Zalgen Labs, LLC, Germantown, MD, USA
d Viral Hemorrhagic Fever Program, Kenema Government Hospital, Kenema, Sierra Leone
e Ministry of Health and Sanitation, Freetown, Sierra Leone
f Polytechnic College, Kenema, Sierra Leone
g Sections of Infectious Disease, Departments of Pediatrics and Internal Medicine, School of Medicine, Tulane University, New Orleans, LA, USA
h Department of Microbiology and Immunology, Tulane University, New Orleans, LA, USA

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A B S T R A C T
Lassa fever is a significant health threat to West African human populations with hundreds of thousands of annual cases. There are no approved medical countermeasures currently available. Compassionate use of the antiviral drug ribavirin or transfusion of convalescent serum has resulted in mixed success depending on when administered or the donor source, respectively. We previously identified several recombinant human monoclonal antibodies targeting the glycoprotein of Lassa virus with strong neutralization profiles in vitro. Here, we demonstrate remarkable therapeutic efficacy using first-in-class human antibodies in a guinea pig model of Lassa infection thereby presenting a promising treatment alternative.

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Lassa Fever (LF) is a major public health problem in Western Africa where hundreds of thousands of infections are estimated to occur annually. For those who present to a hospital for treatment and are referred to a ward specific for the treatment of viral hemorrhagic fevers, the mortality rate approaches 70% (Shaffer et al., 2014). Elevated mortality, environmental stability that favors artificial aerosol infection, and a dearth of medical countermeasures for LF also make Lassa virus a clear biodefense concern (Stephenson et al., 1984). The nucleoside analog ribavirin, convalescent sera, and supportive therapy are the only treatments options currently in use for LF. Safety profile concerns, lack of controlled and randomized clinical studies, and short window of efficacy for therapeutic ribavirin or convalescent sera use in LF patients support the need for development of alternative and robust therapeutic options (Bausch et al., 2010). Single monoclonal antibody therapies against viral agents have recently proven highly effective; however, only one has been approved for use in humans (Mejias and Ramilo, 2008). The 2013–2016 Ebola hemorrhagic fever epidemic in West Africa has, however, potentiated the accelerated development and clinical evaluation of ZMap, a cocktail of three chimeric monoclonal antibodies against distinct epitopes on the Ebola virus glycoprotein (McCarthy, 2014).

Since 2008, the Viral Hemorrhagic Fever Consortium (http://www.VHFC.org) has concentrated efforts at characterizing protective or pathogenic roles of B cells in LF directly from human patients (Robinson et al., 2016). This work has resulted in the derivation of the largest known collection of fully human monoclonal antibodies (huMAbs) from convalescent West African LF patients. Here, we describe the protective efficacy of select LASV glycoprotein specific huMAbs in vivo where a panel of 11 huMAbs targeting the
glycoprotein of Lassa virus (LASV) was evaluated in an outbred guinea pig (GP) model of LF based on their in vitro neutralization profiles.

Optimized bicistronic IgG1 backbone vectors for high level, transient expression in mammalian cells were used to generate huMABs for therapeutic evaluation in vitro and in a GP model of LF. Scaled production of antibodies for in vivo studies is detailed in the supplementary materials. Antibodies for therapeutic studies were formulated in a buffer for injection at >20 mg/mL, and assayed for purity, concentration, identity, and endotoxin levels prior to lot release.

A panel of 125 independently derived LASV glycoprotein-specific huMABs were extensively characterized for binding, epitope grouping, and neutralization potential in vitro (Robinson et al., 2016). Eleven huMABs consistently generated greater than 50% neutralization in a lentiviral entry assay system pseudotyped with the LASV Josiah strain, Clade IV GPC, a lymphocytic choriomeningitis virus (LCMV) backbone pseudotyped with LASV GPC, or in a live LASV plaque reduction neutralization test (PRNT) (Robinson et al., 2016).

Antibodies segregated into two distinct performance clusters of IC50 and IC80 values (Supplemental Table 1, Fig. 1A, B). Neutralizing antibodies (NAb)s 37.2D, 8.9F, 12.1F, 37.7H, 25.6A, 36.1F, and 25.10C clustered in the lowest combined Log10 IC50 (Fig. 1A,) and Log10 IC80 (Fig. 1B,) value ranges. A second cluster of NAbS comprised of 19.7E, 10.4B, 37.2G, and 2.9D recorded significantly combined higher Log10 IC50 and Log10 IC80 values range (Fig. 1A, B). Ten of the 11 huMABs bound mammalian cell-generated LASV glycoprotein immobilized on ELISA plates. The single exception, huMAB 8.9F was identified via a pseudovirus neutralization assay performed as previously reported (Robinson et al., 2016), and did not bind purified glycoprotein in ELISA.

Binding efficacy to different clades of LASV was assessed using flow cytometry to detect the glycoprotein complex (GPC) genes of LASV Josiah (Clade IV), and Nigerian strains NigA19-08 (Clade III) and NigA18-237 (Clade II) which were transiently expressed on the surface of transfected HEK-293T/17 cells for 24 h prior to flow cytometric analysis (FACS). Histograms were generated that display the log fluorescence intensity for each antibody bound to GPC on the surface of live transfected cells. All 11 huMABs bound to HEK-293T/17 cell surface-expressed LASV GPC by flow cytometry (Fig. 1C). Three LASV huMABs (8.9F, 12.1F, and 37.2D) also bound cell surface expressed GPCs from Clade II (strain Nig A18-237) and Clade III (strain Nig A19-08) strains (Fig. 1C, D). These binding profiles suggest that these 3 LASV huMABs may have therapeutic utility across LASV Clades. These data guided the identification and prioritization of leading candidate therapeutic huMABs for in vivo studies.

Animal studies were completed under biosafety level (BSL)-4 biocontainment at the Galveston National Laboratory and were approved by the University of Texas Medical Branch Institutional Laboratory Animal Care and Use Committee. Female outbred Hartley strain GP (~350–400 g. ~5–6 weeks old, Harlan Sprague Dawley, Houston, TX) were acclimatized for ~1 week prior to challenge intraperitoneally (IP) with 1000 plaque forming units of guinea pig adapted (GPA) LASV-Josiah (N = 5/treatment group). The GPA LASV infection model of outbred Hartley GP has been described recently for testing therapeutics against LASV (Safro netz et al., 2015). The advantage of using outbred animals to model human infection is inferred from the higher variability of immune responsiveness inherent in outbred populations allowing for increased countermeasure testing stringency versus those inbred for genetically tractable immune deficiencies which may, in turn, result in reduced predictive efficacy of the treatment in question by only testing a single immune phenotype rather than multiple at one time, as would be present in an outbred test population.

Animals were administered huMABs (30 mg/kg of body weight) IP immediately after challenge (day 0) and on days 3 and 6 post infection (PI). Control animals were not treated. Guinea pigs were monitored for clinical observations, appearance, behavior, activity, and signs of paressis. Clinical scores were determined as Bright, Alert, Responsive = 0, Rough = 4, Sick = 8, Moribund = 12, or Expired = 16 over the course of 28 days after which survivors were euthanized at the study endpoint per IACUC protocol. Animals which were scored rough had roughed fur, delayed responsiveness, and some evidence of weight loss. Sick animals had marked lack of grooming, unstable gait, and clear evidence of weight loss. Blood was collected into EDTA containing tubes on days 7 and 14 and viremia was assessed from separated plasma using classical titration methods as outlined in supplemental materials. Kaplan-Meier survival curves were generated in Prism 5 and survival curves were compared using Log-rank (Mantel-Cox) test (GraphPad Software, La Jolla, CA) for treated groups versus untreated controls for each study (Fig. 2A).

Eleven LASV huMABs tested in a Hartley GP model of LF segregated the antibodies into three distinct protection groups. One group of huMABs, 25.6A, 2.9D, 8.9F, 12.1F, and 37.7H conferred 100% protection and no change in clinical score in GPs. In a second group, 37.2G, 19.7E, and 37.2C protected 80–90% of animals. A third group comprised of 10.4B, 25.10C, and 36.1F, conferred 40, 30, and 20% protection, respectively. An irrelevant recombinant human isotype control (IgG1) antibody did not confer protection (0% survival). From the tested antibodies, no significant differences were observed between protection level and epitope groups; however, more antibodies from each respective treatment group will need to be tested in order to verify the potential importance of any one particular epitope group in regard to protection (Supplemental Table 1).

The combined in vitro IC50 and IC80 data generally correlated well with protection in vivo for the top tier antibodies (100% protection). All but 2.9D neutralized pseudovirus in the nanomolar range, and registered a PRNT IC50 mean of 13.6 nM. Despite conferring 100% protection in GP huMAB 2.9D registered high IC50 values in vitro (Supplemental Table 1). 37.2D and 19.7E generated distinct IC50 values in pseudovirus assays, with the former neutralizing in the low nanomolar range, and the latter at >20 nM. Both antibodies neutralized LASV in PRNT at IC50 ~ 18 nM, and conferred 90% overall protection in GPs. It should be noted that 19.7E and 37.2D antibodies conferred 100% protection yet showed some clinical signs in an initial study (5/5) so a repeat study was performed where 80% (4/5) survived and similar clinical values were observed.

37.2G protected 80% of animals tested, despite high IC50 values in pseudovirus and PRNT assays (16.0 and > 100 nM, respectively), 10.4B neutralized poorly in all assays and conferred only 40% protection in GPs. Surprisingly, the most potent antibody identified in vitro, 25.10C, with subnanomolar activity in all pseudovirus platforms, and 6.24 nM in PRNT, conferred an average 30% (3/10) protection in GPs. In our initial assessment, 25.10C protected 40% (2/5) of GPs, yet only 20% (1/5) in a repeat study. The difference in results could be attributed to inherent immune variability of outbred animals where differences in individual’s susceptibility to the virus contributes to disease course. Importantly, these data indicate that in vitro neutralization assays have value in selecting antibodies with protective efficacy in vivo, but are not necessarily predictive of levels of protection.

Viremia was compared by Kruskal-Wallis test supported by Dunn’s Multiple comparison posttest (Prism 5, GraphPad Software, La Jolla, CA) to detect differences from the control group for time points relevant to onset (day 7) or peak viremia (day 14) as determined from historical data (Geisbert, T.W. unpublished data).
Untreated control animals averaged 3.5 and 4.5 Log PFU/mL on days 7 and 14, respectively. Despite 100% protection at the study endpoint, some animals from treatment group 8.9F or 37.7H, 2.9D, and 25.6A had low level viremia on day 7 or 14, respectively. Treatment groups where 90% protection was afforded (37.2D and 19.7E) had reduced mean viremia titers and minimal clinical score values. Treatment groups with 80% or less survival had comparable mean viremia titers to control animals on day 7, but by day 14 mean viremia was markedly lower than control animals. Groups with 80–90% survival exhibited relatively low mean clinical scores and all remaining treatment groups exhibited concomitant increases in mean clinical scores with decreases in survival per group (Fig. 2B, C, and Supplemental Figs. 1). Endpoint viremia was not determined for these studies as survival was the primary metric of interest, though all surviving animals demonstrated no clinical signs to indicate disease was present at study endpoint 28 days PI (Fig. 2B and Supplemental Fig. 1).

Lassa fever is a major public health threat in West Africa. Recent importations of cases of LF to non-endemic regions of the world illustrate the global public health threat (Amorosa et al., 2010; Haas et al., 2003; Hirabayashi et al., 1989). Currently, no approved LF vaccines or therapeutics exist, yet there is a clear public health need in endemic regions. Further, LASV has been categorized as one of the World Health Organization’s top 10 emerging pathogens and has thus created increased demand for medical countermeasures (WHO, 2016). Off label use of Ribavirin can be partially effective in the treatment of acute LF; however, it has a narrow window of efficacy, generally within 6 days following the onset of fever and is often associated with marked side effects due to dosing regimen (Bausch et al., 2010). Unfortunately, the drug has not demonstrated great efficacy in treating advanced LF (Bausch et al., 2010).

The current studies present a novel alternative for treating LF infections that relies on the use of fully human antibodies derived from convalescent donors. Early studies employing convalescent plasma to treat active LF infections met with mixed success; this was likely due to unreliable titers of protective antibodies in
donors’ blood or antibody specificities that were not well matched against circulating LASV strains (Cummins et al., 1991; Jahrling et al., 1985; Leifer et al., 1970). Here we show that the majority of antibodies administered either individually or in combination prevented development of active disease in GP when administered soon after infection. These studies demonstrate the in vivo potential of these antibodies and merit further preclinical development of huMAbs as mono-therapies or cocktails for pre-exposure prophylaxis or post-exposure treatment in the gold standard animal model for LF, non-human primates. This technology may be a particularly relevant treatment for high risk scenarios such as caregivers working directly with LF patients or those who have otherwise become exposed to the virus in the community. In the absence of approved vaccines, safe and effective immunotherapeutics would provide a superior prophylactic and post-exposure treatment alternative, with well tolerated profiles, and likely few metabolic side effects.

The ongoing outbreak of LF in Nigeria and neighboring Benin, endemicity of the disease in Sierra Leone, Guinea, and Liberia, potential for geographic expansion (Fichet-Calvet and Rogers, 2009) and threat to national security if developed as a bioweapon should prioritize development of a new and safe class of Pan-Lassa virus therapeutics (Ibekwe, 2012).

Conflict of interest

RWC, CEM, JBG, MMR, MLH, AG, MM, DSG, JER, and TWG declare that they have no conflicts of interest. LMB and RFG are co-founders of Zalgen Labs, a for profit biotechnology company.

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Presentation history

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.antiviral.2016.08.012.

References


