The Use of Human Monoclonal Antibodies as an Immunotherapeutic Against Lassa Fever

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Lassa virus (LASV) is a single-stranded RNA virus belonging to the *Arenaviridae* family, which causes an acute viral hemorrhagic disease known as Lassa fever (LF)\(^1\)-\(^3\). LF was first described in the 1950’s, but the causative virus was not isolated until 1969\(^1\),\(^4\). It is now known that LASV is endemic throughout several West African countries, including Ghana, Sierra Leone, Nigeria, Guinea, Liberia, and Benin, and is thought to be present in other countries as well\(^1\)-\(^3\),\(^5\). LASV infects more than 100,000 patients every year, and the case fatality rate of hospitalized LF patients can exceed 50\%\(^2\),\(^3\),\(^6\). LF is a zoonotic disease, and the *Mastomys natalensis* rat acts as a natural reservoir of LASV\(^1\),\(^3\). As such, infected animals do not become ill, but they do shed virus in both their urine and feces\(^1\). Human transmission occurs when infected rat waste comes into contact with food or other household items; however, human to human transmission is also known to occur\(^1\),\(^6\).

LASV has an incubation period of anywhere from 6-21 days\(^3\). Interestingly, it is asymptomatic in ~80\% of cases\(^1\). However, observed symptoms are generally non-specific, and include an acute onset of fever, weakness, and malaise\(^1\). Symptoms may also manifest as a headache, sore throat, muscle pain, chest pain, nausea, vomiting, diarrhea, cough, and abdominal pain\(^1\). Severe cases may result in low blood pressure, facial swelling, fluid in the lung cavity, and bleeding from the mouth, nose, vagina, or gastrointestinal tract\(^1\). There are two generally accepted outcome predictors for LF: serum aspartate aminotransferase (AST) levels and viremia levels\(^7\). High case fatality rates are associated with AST levels at ≥110 IU per litre or viremia at levels ≥10\(^{3.1}\) TCID\(_{50}\) per millilitre\(^3\),\(^7\).

Because LF is primarily endemic in remote, rural areas, the development of proper therapeutics has proven difficult, as they must be cheap, easy to administer, and have no serious side effects\(^7\). As such, there is currently no vaccine available, and besides supportive therapy, there are currently only two candidate therapeutics: ribavirin and convalescent plasma\(^2\),\(^5\),\(^8\).

Ribavirin, a nucleoside compound composed of ribose and triazole, has been shown to have an inhibitory effect on the replication of LASV *in vitro*\(^2\),\(^7\). Specifically, it is thought to be a competitive inhibitor of guanosine in the 5’ capping of viral mRNA\(^7\). However, recent *in vivo* studies have shown that ribavirin does not reduce viremia, and instead reduces AST levels, likely resulting in a reduction of hepatic cell damage instead\(^2\). Regardless of the exact mechanism of action, *in vivo* assays conducted in the 1980’s found that ribavirin greatly reduced the mortality
rate when given intravenously within the first 6 days of illness, with case fatality rates dropping from 55% to 5% upon administration \(^5,7\).

Research concerning the use of human convalescent plasma as a potential LASV therapeutic has had varying success, and has resulted in inconsistencies. Some studies have shown that the administration of convalescent plasma does little to improve the outcome of patients infected with Lassa fever \(^2,7,9,10\). Alternatively, there are reports of patient cohorts which have shown that the administration of convalescent plasma was effective in reducing mortality rates \(^5,7,9,11\). From a genetic standpoint, the neutralizing effect of antibodies on LASV has been difficult to characterize for several reasons, including viral mechanisms designed to shield LASV, the seeming lack of a humoral response in LASV-infected patients, and the incredible range of sequence diversity displayed by LASV.

The LASV genome consists of two parts: a small (S) segment and a large (L) segment \(^3,12\). The small segment encodes the glycoprotein precursor (GPC), which is post-translationally cleaved into two peptides, GP1 and GP2, which make up the transmembrane protein \(^3,6,12\). The GPC is the sole antigen present on the viral surface, and is the primary target for the humoral immune response \(^13\). As such, it is important to note that mutations are likely to accumulate within the epitopes of the GPC, resulting in escape mutants \(^6\). Further, the glycan coat of LASV has recently been implicated in shielding the GPC from antibody binding, and thus their neutralizing ability \(^12,14,15\).

Despite these viral attempts to shield LASV from neutralizing antibodies, it is known that LASV-infected non-human primates (NHP) develop antibodies to GPC relatively quickly, with detectable IgM titres seen at 9 days post-infection, and IgG titres seen at 12 days post-infection \(^3,6\). In LASV-infected humans, IgG and IgM responses are seen, but in smaller amounts than what would be expected throughout a normal humoral response to infection, which is likely due to the aforementioned structural properties of the GPC \(^3,12\). Binding of the LASV glycoprotein by antibodies is thought to either i) prevent the binding of the LASV glycoprotein to the cellular receptor \(\alpha\)-dystroglycan, ii) prevent fusing of the viral envelope with the host cell membrane through glycoprotein binding, or iii) both of the above \(^5\). Recent biochemical analysis of antibodies have suggested that they neutralize LASV by inhibiting conformational changes required for entry \(^13\). Overall, the humoral immune response has proven difficult to characterize in LASV infection \(^12\).
To further complicate LASV immunotherapy research, sequencing of clinical samples, *Mastomys natalensis* samples, and viral laboratory isolates have shown high levels of LASV diversity, with strain variation of up to 32% and 25% for the L and S segments, respectively. Additionally, LASV sequences clustered into four clades, which are dependent on region and are not host-specific. This has spurred the idea that it may prove helpful to develop vaccines and therapeutics in a country-specific manner, in order to target the most reserved regions within each of the known clades.

Despite these inherent difficulties involved in the administration of convalescent plasma as an immunotherapy for LF, infected patients are frequently treated in this way, with varying success. There are seven documented occasions between 1970-1976 where convalescent sera was administered to patients infected with LASV. These cases were split almost equally in terms of success, with four patients making a full recovery, and three patients succumbing to LF.

One of these cases occurred in 1969, when an individual researching LF in a laboratory setting became infected and was hospitalized 6 days after the onset of symptoms. On day 9 post-symptom onset, the patient was administered 500 mL of plasma obtained from an individual in the 16th week of convalescence from LF. The obtained convalescent plasma was known to have a high antibody titre, and was able to neutralize $10^2$ TCID$_{50}$ of LASV. It appears that the transfusion of convalescent plasma was helpful in treating LF in this case, as the patient made a full recovery following sera administration. However, it is difficult to ascertain whether the administration of sera was the sole contributor of health, or if the patient would have recovered regardless.

Alternatively, in 1975, a 22-year old patient was admitted to the hospital with LF several days after symptom onset. Nine days after hospital admission, she was administered 600 mL of plasma. However, the plasma seemed to have no effect, as she died the following day. It is important to note that in all three unsuccessful cases of sera administration, the plasma was not administered until well after the onset of symptoms, which was thought to possibly have had an impact on its success. The details and outcomes of each of these seven cases are summarized in Figure 1. At the time, it was believed that both the antibody titre, as well as the time of administration following symptom onset was important. However, at this point in history, there was no satisfactory virus-neutralizing antibody test, and instead researchers simply
recommended the administration of convalescent sera as soon as possible following positive LASV identification.

Further research occurred in the 1980’s, when therapeutic trials using convalescent plasma as a treatment for LF took place in rural hospitals in Sierra Leone. Researchers found that the case fatality rate in patients treated with convalescent plasma did not significantly differ from the untreated group, and was significantly higher than patients treated with intravenous ribavirin. Additionally, researchers found that the case fatality rate in sera-treated patients did not differ dependent on the number of doses given. These results seemed dismal, and further underlined the fact that in human cases where sera was administered, it is impossible to determine whether patients truly benefitted or would have recovered regardless. The results from this study are further summarized in Figure 2.

Also in the 1980’s, Jahrling et al. conducted research concerning the use of convalescent plasma to treat LF in cynomolgus monkeys. The researchers found that infusions of high-titred Lassa-immune monkey plasma with a log neutralization index ≥2.6 administered on day 0, 3, and 6 post-infection, resulted in relative protection. Specifically, protection was conferred in 87.5% of animals administered 1 ml/kg of convalescent plasma with a log neutralization index of 4.1. Interesting, all animals administered 1 ml/kg of convalescent plasma with a log neutralization index of 2.6 succumbed to LF, but the administration of the sera did seem to delay the disease course. However, when animals were administered a higher dose of 3 ml/kg of convalescent plasma with a log neutralization index of 2.6, 100% protection was conferred. These results are summarized in Figure 3, but seem to indicate that the log neutralization index of the administered convalescent sera is incredibly important, along with a proper dose.

In a separate study, Jahrling et al. wished to study the neutralizing antibody titre present in the plasma of LF-convalescent patients, as prior research seemed to indicate that a log neutralization index of at least 2.0 was necessary for passive immunization. However, in the tested patients only minimally protective titres were seen, and it was found that only ~30% of patients had a log neutralization index above 2.0. Moreover, it was observed that the high titres necessary for LF treatment only occurred approximately 7 months post-infection, and only in certain patients. The realization that sera obtained from a large majority of LF-convalescent patients may not be suitable for inducing protection may help explain the varying success seen over time when administering convalescent plasma to LF-infected patients.
In 2014, the United States launched The Global Healthy Security Agenda, which along with funding from other G7 nations, aims to strengthen international prevention, detection, and response to infectious diseases. This resulted in a push in recent years to develop proper therapeutics and vaccines for many infectious diseases worldwide, including LF.

One study used human monoclonal antibodies (huMAbs) isolated from convalescent sera to treat LASV-infected guinea pigs, which proved incredibly successful and underlined the potential of convalescent plasma as a LF therapeutic. Researchers characterized 125 individual antibodies, to determine binding, epitope grouping, and neutralization potential in vitro, using a lentivirus system pseudotyped with the LASV glycoprotein from clade IV. From these 125 possible antibody candidates, 11 were identified as consistently resulting in greater than 50% neutralization.

To further test the neutralization ability of these antibody candidates, guinea pigs were challenged with LASV, and immediately administered huMAbs intraperitoneally, as well as at 3 and 6 days post-infection. At 28 days post-infection, all surviving animals were euthanized. Five of the tested antibodies resulted in 100% protection in the LF-infected guinea pigs. Additionally, 2 of the tested antibodies conferred 90% protection in the animals. These in vivo results seem incredibly promising in terms of future, alternative treatments for LF, and helped spur modern research in this field. The results obtained by Cross et al. are further summarized in Figure 4.

The most recently conducted, and most promising research concerning the use of huMAbs from convalescent plasma as an immunotherapeutic for LF was conducted in cynomolgus macaques. Researchers chose five LASV-neutralizing huMAbs which were deemed promising, based on their success in the aforementioned guinea pig study, along with their cross-reactivity to all four LASV clades and their neutralization abilities in vitro. Macaques were challenged with LASV, and the chosen huMAbs were immediately administered as a single-antibody treatment. Additional treatments were also administered at 4 and 8 days post-infection for four of the antibody treatments, while the fifth antibody treatment was administered at 5 days post-infection. While one animal had detectable infectious virus and required euthanasia, it was found to be due to an escape mutant, which had a single amino acid mutation in the GP1 subunit. Besides the escape mutant, all of the single-antibody treatments resulted in 100% protection (Figure 5).
Interestingly, treatment with a combination of three of the chosen huMAbs was able to neutralize the escape mutant virus \textit{in vitro} \textsuperscript{5}.

Researchers also studied the effect of cocktail therapies, or treatment with a combination of huMAbs. Cocktail therapies were administered immediately upon LASV challenge, as well as at 3, 6, or 8 days post-infection. Each of the cocktail therapies resulted in 100% protection, with each of the treated animals surviving until the study endpoint (Figure 6) \textsuperscript{5}. More impressing, the effectivity of these cocktail therapies was also studied when delayed. Prior to the administration of the cocktail therapies, each of the animals were viremic, with therapies not administered until 3, 6, and 8 days post-infection, respectively. Even when delayed, treatment with cocktail therapies resulted in 100% protection, with all clinical signs and illness being resolved for each of the LASV-infected animals \textsuperscript{5}. This use of antibody cocktails ensures that LASV will be less likely to develop resistance, and seemed to confirm the long-held idea that huMAbs can indeed confer protection to LF-infected patients.

The future of LF immunotherapy seems promising. However, further research must be conducted using convalescent plasma. It has been postulated that previous research conducted with convalescent sera was unsuccessful due to the presence of antibodies which were poorly matched to the circulating LASV, or a low log neutralization index \textsuperscript{5,16}. However, modern research seems to have overcome these shortfalls. Moreover, the use of antibody cocktails has shown incredible success in preliminary studies, and should be further studied in a nonhuman primate model, before hopefully moving into human trials. With the recent successes in the field of convalescent plasma as an immunotherapeutic against LF, it is hopeful that LF will soon be overcome by modern science, and will no longer pose a threat to the thousands of individuals infected each year.
A summary of all 7 cases in which convalescent plasma was administered as an immunotherapeutic against LF from 1969-1976. The cases discussed here are #1 and #6. Taken from Clayton 9.
Results showing that the case fatality rate in patients treated with convalescent plasma in Sierra Leone in the 1980’s did not significantly differ from the untreated group, and was significantly higher than patients treated with intravenous ribavirin. Taken from McCormick et al. 7.
A summary of Jahrling et al.’s results showing that a high log neutralization index ($\geq 2.6$), as well as proper dosing, is vital to the successful administration of convalescent plasma in LF-infected cynomolgus monkeys. Taken from Jahrling et al. 16.
Graph representing the fatality rate in guinea pigs infected with LASV, followed by treatment with human monoclonal antibodies. 100% protection was conferred following treatment with 5 of the chosen antibodies. Taken from Cross et al. 8.
Graph showing that all of the human monoclonal antibodies administered to the cynomolgus macaques by Mire et al. resulted in protection from LF, other than a single escape mutant. Taken from Mire et al. 5.
Figure 6:

Summary of each of the cocktail therapies administered to LF-infected cynomolgus macaques by Mire et al., resulting in 100% protection. Taken from Mire et al. ⁵.
Graph showing the circulating viral load for each of the animal cohorts who received delayed cocktail therapies. Protection was conferred in 100% of the animals. Taken from Mire et al. 5
References:


