

Lassa Fever Live Attenuated Recombinant Vaccine

Epidemiology:

Almost forty years ago in 1969, the first discovery of Lassa fever in Nigeria presaged an inevitable epidemic in the years to come. The Lassa virus is known to have originated from several unknown species of rodent from the genus *Mastomys*. Their frequent reproductions results in wide distributions of ubiquitous offspring spreading across West Africa: Nigeria, Guinea, Liberia, Sierra Leone, and perhaps more. The other major contributing factor stems from indirect and direct human-rodent contacts. Rodents' excretions (urine and feces) make suitable vectors for transmission of the virus, and human consumption of contaminated food make one as well. Additionally, the direct contact sometimes results from rodents being used as a food source. These variables lead to another mode of transmission: human-human contact. A non-infected individual may contract the virus through contact with blood, tissues, and other excretions of bodily fluids from infected individuals (Lassa Fever, CDC).

Collectively, the mortality-morbidity statistics often yield high crude figures. Annually, infection rate approximates between 100,000 to 300,000 along with 5,000 related deaths. Hospitalized individuals with Lassa fever have 15-20% mortality rate (Lassa Fever, Wikipedia). The morbidity increases with infected third-trimester fetuses—95% death rate. Among the many challenges in studying such an endemic lies the difficulty in diagnosing infected individuals. Approximately 80% of infected individuals do not show symptoms for analytical observations. In those who present observable symptoms within one to three weeks of infection, the symptoms may vary (Lassa Fever, CDC): retrosternal pain (chest wall), sore throat, cough, diarrhea, fever, back pain, abdominal pain, vomiting, mucosal bleeding, conjunctivitis, facial swelling, hearing loss and encephalitis.

Unfortunately, there are currently no effective vaccines. However, several preventative measures can be taken to reduce the mortality-morbidity rates. The implementation of several methodologies renders Lassa virus detection more effectively. Enzyme-linked Immunosorbent Assays (ELISA), immunohistochemistry, and reverse transcription-polymerase chain reaction (RT-PCR) are available tools for clinical diagnoses and research (Lassa Fever, CDC). Additionally the unprecedented antiviral drug, Ribavirin, proves its efficacy in the early stage of infection disrupting virus metabolic activities and duplication of genetic contents. The more practical and cheaper method entails decreasing exposures to infected rodents and humans. Rodents will colonize homes with accessible food sources. Clinical settings necessitate proper attire, gloves, and masks to provide barriers against nosocomial infections (Lassa Fever, CDC).

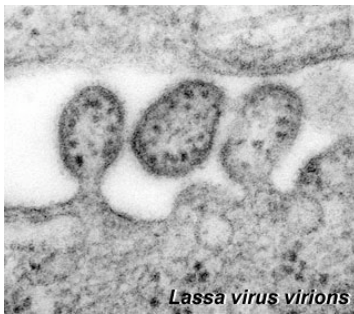
Disease Mechanism:

The Lassa virus most commonly enters the body through the respiratory tract mucosal membranes (specifically the nasal lining) in the form of microscopic particles derived from rodent fecal matter. Upon entering the body, the virus is recognized by macrophages, which bind to the glycoprotein surface markers of the virus and become activated. The innate immune response is initiated by the macrophages (in early infection, 5-7 days) and inflammation occurs. The virus rapidly spreads throughout the body, infecting nearly all body tissues. However, it is not this rapid proliferation of the virus that causes its extensive physical damage, it is the inflammatory response invoked

by activation of macrophages that causes the massive capillary leakage, febrile symptoms, and internal hemorrhage characteristic of the Lassa virus. The virus reaches an extreme infectious stage because of its ability to disarm the adaptive immune response through inactivation of dendritic cells. Because the dendritic cells cannot function properly, the body is unable to mount an adaptive response to the pathogen, and the body continues to employ its innate responses to rid the body of the virus. What the virus actually does to the dendritic cell is still under investigation, but it has been shown experimentally that when infected by the Lassa virus, dendritic cells fail to release important cytokine and chemokine messengers (such as TNF- α , many Interleukin molecules) as well as the co-stimulatory molecule CD86. Discovering the method by which the virus inactivates the dendritic cell may open up new methods of treatment of the Lassa Virus.

Pathogen Structure:

The Lassa virus is an RNA virus that contains one strand of ambisense RNA that is divided into two segments and packaged inside of a lipid envelope. Ambisense viruses contain two types of RNA, negative and positive. Positive RNA is directly translated into protein, while negative RNA must first be transcribed into positive RNA (similar to how DNA is transferred into mRNA) and then translated into a protein. Lassa is of spherical



structure and is roughly 80-20 nm in diameter with a granular appearance when looked at under an electron microscope (Salvato 2005). The most significant structure on the Lassa pathogen is the nucleocapsid protein, which also comprises the largest fraction of the coding genes in the virus' RNA. The envelope contains a glycoprotein which is comprised of membrane embedded glycoprotein-2 ionically bound to extracellular glycoprotein-1 (Salvato 2005).

Subject Population:

Lassa virus is carried by small rodents, and is transferred through their fecal matter to humans. Because Lassa's vector can so easily migrate from one country to another (by means of human transportation on boats, trains, trucks, etc), it is possible for Lassa to spring up anywhere (which increases its appeal as a biological weapon). However, it is most prevalent in many West African countries such as Liberia and Nigeria. The focus of this vaccine is on military persons and travelers, many of whom have to be passengers aboard boats infested with rodents, to countries in which Lassa has a prevalence ranging from meager to significant. It is important to be able to properly protect these groups of people from contracting the Lassa virus, and transporting it to the United States, where it undoubtedly would have a catastrophic effect.

Vaccine:

Lassa virus (LASV) belongs to the family *arenaviridae* and is sub classed into the Old World group of arenaviruses. The Old World group contains three closely related viruses Mopeia (MOPV), Mobala (MOBV), and Ippy (IPPYV), MOPV has the most RNA homology to LASV. With the exception of LASV, the other viruses are non-pathogenic to humans and primates and only seem to affect rodents (Lukashevich 2005). *Arenaviridae* all have a characteristic genome containing 2 single stranded RNA pieces,

one Short (S) which encodes surface glycoproteins (GP1 and GP2) and nucleoproteins (NP), and a Long (L) strand that encodes polymerase and a zinc-binding protein.

MOPV was chosen for the recombinant vaccine due to its close sequence homology to LASV, it behaves like a weakened form of LASV (Fisher-Hoch 2005). Lukashevich et al. (2006) has had success with a LASV/MOPV recombinant vaccine consisting of the L RNA segment from MOPV and S RNA segment from LASV. Research has shown that the L strand is more pathogenic (Lukashevich 2005) therefore using the weaker virus L strand should reduce virulence. One intramuscular injection of the LASV/MOPV live recombinant vaccine should be sufficient for those with healthy immune systems.

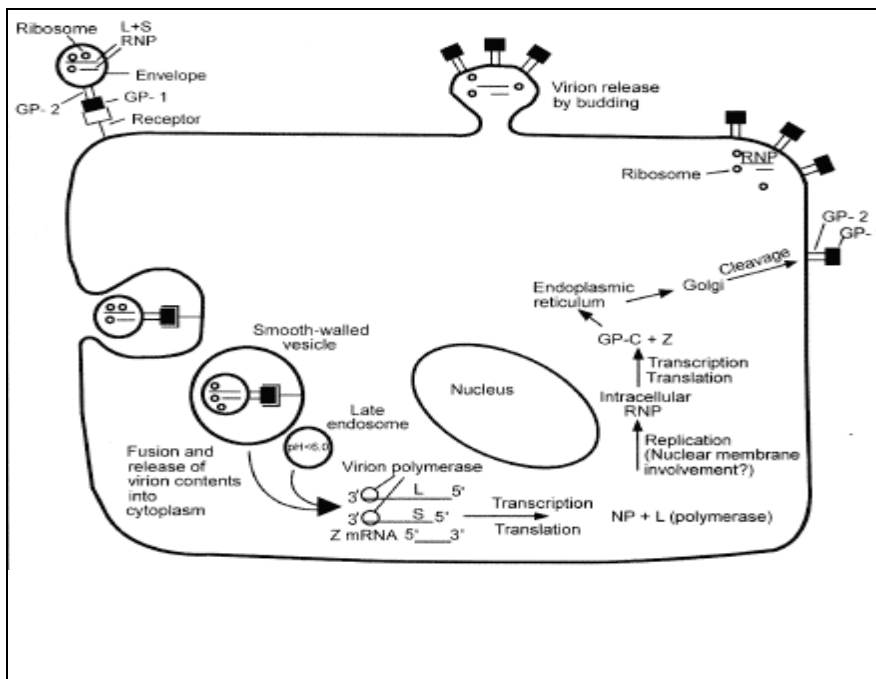


Image 2 Diagram of LASV Replication within a cell (Lukashevich 2006)

Innate Immune Response:

Following vaccination the innate immune system will respond first to the recombinant virus. Macrophages binding antigen (GP1, GP2, and NP) on TLR receptors will release cytokines and chemokines IL-1, TNF- α , IL-6, IL-8, and IL-12 to induce inflammation. TNF- α aids in increasing adhesion molecules on walls of capillaries causing neutrophils to stick and migrate out. TNF- α , IL-1 and IL-2 have long range effects as well and can stimulate the acute phase response proteins in the liver, one of these being C-reactive protein. The C-reactive protein when bound to pathogen activates complement and opsonization of pathogen.

When cells are infected with virus they will release INF- α and INF- β which will bind to nearby cells and tell them to stop cell replication in an attempt to stop the virus from replicating. INF- α and INF- β also stimulate APC's to display more MHC class I to up antigen presentation to Tc cells in lymph nodes.

INF- α , INF- β , and IL-12 released by infected cells will signal Natural Killer cells to infection site. NK cells look for non-self proteins and if one is found they will release

perforins and granzymes which tell the infected cell to undergo apoptosis. NK cells are very important in containing a viral infection while adaptive immunity is being developed. Once the pathogen is attached to an antigen presenting cell, such as a dendritic cell or macrophage, it will be taken to a lymph node and the adaptive immune system will activate.

Adaptive Immune Response:

Dendritic cells will ingest pathogen, process it and display polypeptide on MHC Class I and Class II to naïve T-cells in lymph nodes. Infected macrophages can also present pathogen to T-cells but are not as effective. In the lymph nodes naïve T-cells sample class I and class II MHC on many cells passing through lymph, when one binds tightly to the T-cell receptor (TCR) it waits for a secondary signal binding of B7.1 or B7.2 on DC to CD28 on T-cell before activating. Once co-stimulation has occurred the T cell will release IL-2 and bind IL-2 to cause the selected T-cell to replicate and divide. IL-2 is a T-cell growth factor. If the naïve T-cell binds MHC Class I it will mature into a cytotoxic T-cell, MHC class II bound T-cells mature to helper T-cells. The mature Tc and Th cells will migrate from lymph to site of infection to fight pathogen infected cells. Some Th cells will remain in lymph nodes and aid B-cells in becoming activated against GP1, GP2, and LP.

The Tc and Th cells find the infection site by adhesion molecules lining the capillaries at the site. Tc cells look for cells expressing MHC class I pathogen peptide. When bound they will release effector molecules to destroy the cell. Perforin makes holes in the target cell membrane; granzymes (proteases) will enter the infected cell and trigger apoptosis. Tc cells express a Fas ligand, if bound to a Fas receptor on an infected cell, apoptosis will occur. Once the cell has been instructed to die the Tc cell moves onto another infected cell and repeats the process. Tc cells also release IFN- γ , TNF- α , and TNF- β cytokines to signal to near by cells to stop replication if infected.

In the lymph nodes Th cells are further divided into two classes Th1 and Th2 the distinction comes from the cells they act on. Th1 cells activate macrophages and Th2 cells activate B cells and the humoral immune response. Due to a virus pathogen we the researchers do not expect to have Th1 cells.

Humoral Immune Response:

The last phase in fighting infection is the humoral response by B-cells. When Th cells bind to Class II MHC on dendritic cells with their TCR, the macrophage releases IL-2 a cytokine that causes the Th cell to activate. Once activated the Th cell will release its own cytokines that create clones of the Th cell. A B-cell will bind antigen epitope of GP1, GP2 or NC to its IgM receptor causing the antigen to be engulfed by the B-cell. Once inside it will be processed and displayed on B-cell surface on MHC Class II proteins. Cloned Th cells can now bind to this MHC Class II protein displayed on B cell surface, binding of B-cell co-receptor CD20 on B cell by CD40-Ligand on Th cell gives the co-stimulatory signal to the B-cell. Once the antigen is bound to the Th TCR it will cause Th cell to release cytokines IL-4, IL-5, and IL-6 which tell the B-cell to make its own clones and proliferate.

Differentiation occurs in the germinal center within lymph tissue where somatic hypermutation will alter the V region of B cells; only B cells with high affinity for the

antigen will be positively selected for. The various interleukins secreted by Th cells play an important role in isotype switching from IgM to IgG. Isotype switching also occurs within the germinal center and experiments show that when exposed to the cytokines from Th cells, the transcription will switch to a different heavy chain C gene either gamma, or epsilon. Eventually a single exon sequence is created consisting of the antigen specific variable region and differing constant regions resulting in different types of antibodies that all bind antigen. The cloned cells will leave the lymph node and go into bone marrow to differentiate into memory cells or antibody secreting plasma cells which are basically antibody factories due to having extensive ER and many ribosomes. The antibodies can either bind antigen and neutralize it or bind and signal to macrophages to destroy it, or activate complement to opsonize the pathogen. Having memory B cells enables the patient to form a quick immune response next time they are exposed to LASV.

Following immunization the patient will have LASV/MOPV antigen specific memory Tc cells, memory B cells, and IgG antibodies giving them immediate immune response should they encounter the pathogen.

Description of Immunity Assessment:

Serum samples will be taken from test subjects 2 weeks prior to the administration of the experimental vaccine or placebo. In natural occurring cases of lassa fever the innate immune response is initiated by the macrophages in early infection, (5-7 days) and inflammation occurs, therefore the next serum sample will be taken 5 days after the injection. The titer of IgG and IgM antibodies taken at day five is not expected to be high because the adaptive immune system has not had the time to respond. Serum samples will then be collected at day 14 and every 14 days following until six months from the administration of the experimental vaccine. After six months serum samples will be collected regularly every 28 days for six additional months; titers of IgG and IgM antibodies are not expected to fluctuate dramatically in this time period. After the first year serum samples will continue to be taken every 3 months to ensure the efficiency, longevity and effectiveness of the experimental vaccine.

It is important to measure the presence of IgM and IgG antibodies made to the vaccine because that is what will determine the overall efficiency of the vaccination. Both IgG and IgM anti-Lassa antibodies can neutralize the antigen. Production of these antibodies will help prevent a natural lassa virus antigen from binding and infecting cells more specifically infecting dendritic cells. Infecting dendritic cells is a virulence factor of lassa virus which prevents important cytokines from being released. Protecting dendritic cells from the infection will substantially increase the effectiveness of the adaptive immune system.

ELISA Testing:

To measure antibodies made to the recombinant vaccine an indirect ELISA will be done using the recombinant antigen consisting of the L RNA segment from MOPV and the S RNA segment from LASV. To detect IgG antibodies a patient's serum will be added to a coated Lassa virus specific antigen microtiter dish. The amount of bound antibody will be measured using either a directly labeled antigen or an antigen that is detected with a labeled monoclonal antibody (Emmerich 2006). The labeled monoclonal antibody that will be used is anti-Lassa mouse gamma chain monoclonal antibody. To detect the presence of IgM in a patient's serum the enzyme labeled antibody will be an

anti-human mu chain monoclonal antibody. The anti-immunoglobulin conjugates that have been applied for the ELISA technique have shown to be effective in detecting both IgG and IgM (Emmerich 2006). The negative control will not contain the antigen coating and the positive control will add a known positive serum to the well plate. To quantitatively measure the amount of antibody present in a patient's serum a spectrophotometer will be used.

Flow Cytometry:

Memory T cells mediate rapid recall functions, characterized by immediate production of effector cytokines such as IFN- γ and TNF- α , and rapid entry into the cell cycle (Farber 2007). CD4⁺ memory cells are crucial for long-term protection as well as a successful vaccine (Lukashevich 2006). Using a tetramer assay we detect the presence of antigen specific T cells. We use Lassa peptide bound to MHC I to stimulate CD8 Th1 cells. The MHC tetramer only binds to our specific T cell responding to that peptide.

For the Flow cytometry we will administer the vaccine and the results will be taken 2 weeks from the initial injection. Our second dose will be administered approximately 7-10 days after the 2 weeks has elapsed from the initial dose. We will compare results and determine the amount of CD8 T cells.

We measure the concentration of CD8 T cells from our inoculated patients' serum with flow cytometry. For our purposes we use a fluorescent dye attached to tetramer peptide MHC I which will be tagged with fluorescein isothiocyanate (FITC) for CD8 antibodies which would be incubated with T-cells with direct immunofluorescence. Our mixture of cells and antibodies will pass through the flow cytometer. The measurement of light is taken per cell as they intercept the light source which converts into electrical pulses that proceed on through optical filters and detectors. When the cells pass through the detector they emit a fluorescent light caused by becoming excited when passing through the light beam. The amount of light that is scattered is measured and used to interpret the data which will be displayed as dots. The scattered light is the results of the light beam from the flow cytometer making contact with the cell. The size of the light scattered is directly correlated to the size and shape of the cell.

Detection of the levels of the fluorescent color would give a measure of how many CD8 Tc cell was present in the original mixture. Our analysis of our results would demonstrate the efficacy of our vaccine if detection of the fluorescein isothiocyanate labeled CD8 antibody were found to be abundant. Most of the Tc cells will die but some of them will continue on as memory cells to respond to the antigen should it reappear again. Practically every cell in the body has class I MHC molecules, so CD8 Tc cells are not limited in what they can attack. Based on the count of CD8 molecules you can determine that the vaccine produced enough CD8 T cells to produce immunity.

References:

- 1) "Lassa Fever." Wikipedia. Feb. 2007.
- 2) "Ribavirin." Wikipedia Mar. 2007. < <http://en.wikipedia.org/wiki/Ribavirin>>
- 3) 7. "Lassa Fever." Center for Disease Control Feb. 2007.
<<http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/lassaf.htm>>
- 4) Borio Md, Luciana, and Et All . "Hemorrhagic Fever Viruses as Biological Weapons." *Journal of the American Medical Association*. Vol. 287 (2002): 2391-2405.
- 5) Emmerich P, Thome-Bolduan C, Drosten C, Gunther S, Ban E, Sawinsky I,

- 6) Farber, D.L. and Ahmadzadeh, M. 2002. Dissecting the complexity of the Memory CD4 T cell response. *Immunologic Research*. 25: 247-59.
- 7) Fisher-Hoch, S. P., McCormick, J. "B. Lassa fever vaccine". *Expert Rev. Vaccines* 2004, 3:103-111.
- 8) Lukashovich, I. S., et al. "A live attenuated vaccine for Lassa fever made by reassortment of Lassa and Mopeia viruses". *Journal of Virology* 2005, 79:13934 – 13942.
- 9) Lukashovich, I. S., Patterson, J. "Reassortant ML29 vaccine protects animals against challenge with heterologous strains of Lassa virus". ASM Biodefense Research Meeting. February 15-18, 2006, Washington, DC. 2006, Program & Abstracts Books: page 65.
- 10) Lukashovich, S. Igor. 2006. "Lassa Virus Genome". *Current Genomics*. 7(6): 351-379
- 11) Mahanty, Siddhartha, Karen Hutchinson, Sudhanshu Agarwal, Michael McRae, Pierre E. Rollin, and Bali Pulendran. "Cutting Edge: Impairment of Dendritic Cells and Adaptive Immunity by Ebola Dna Lassa Viruses." *Journal of Immunology* (2003): 2797-2801. Feb. 2007.
- 12) Peters, C J., C T. Liu, G W. Anderson Jr, J C. Morrill, and P B. Jahrling. "Pathogenesis of Viral Hemorrhagic Fevers: Rift Valley Fever and Lassa Fever Contrasted." *US Army Medical Research Institute of Infectious Diseases*. 1989.
- 13) Salvato, Maria S. "Lassa Fever: a Research Prospective." *Biodefense: Risk, Reality, and Solutions*. 2005. New York Academy of Science.
- 14) Schmitz H. "Reverse ELISA for Ig G and IgM antibodies to detect Lassa virus infections in Africa". *Journal of Clinical Virology*. Vol. 37, Aug. 2006. Pgs. 277-281.