Session 6: Further Research

Prioritizing exploratory research objectives to facilitate vaccine development

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Research Priorities

WHO Target Product Profile: LASV Vaccine
“WHO considers that the highest priority for development is for preventive use”

Define Virus Diversity
- Gene sequencing/reverse genetics
- Develop standardized assays to detect all strains

Develop/Characterize Animal models
- Identify immune correlates
- Compare protective efficacy
- Measure safety
Define Virus Diversity
research prioritization considerations

- LASV has a high level of nucleotide sequence diversity and clusters based on geographic location rather than year of isolation

- Genomic evidence supports the idea that the majority of infections are the result of multiple independent spillover events occurring from distinct reservoir populations with limited movement

- The current diagnostic tests are biased toward strains for which the assays were designed to target; consequently,
  - most detected cases are from patients admitted to specialized LF healthcare facilities, like Kenema General Hospital in Sierra Leone and Irrua Specialist Teaching Hospital in Nigeria
  - it is likely that mild cases of LF are not detected or misdiagnosed in countries and regions where these specialized centers and active surveillance programs do not exist
Define Virus Diversity
research prioritization considerations

Are there differing pathogenicities associated with various strains of LASV?

Disease progression of Lassa virus (LASV)–infected cynomolgus macaques infected with the Malian isolate (Soromba-R) demonstrated prolonged time to death and decreased lethality compared with animals infected with traditional LASV strains from Sierra Leone (Josiah) or Liberia (Z-132).

Define Virus Diversity
research prioritization considerations

Are rodents infected with LASV in regions that have not reported Lassa fever?

Extent of Lassa fever

Predicted geographical distribution of Mastomys natalensis

Animal Models
research prioritization considerations

- The most common small animal model for LASV is Strain 13 (inbred) guinea pigs

- Strain 13 guinea pigs are not readily available
  - Support for a commercially available, characterized colony would facilitate LASV animal model studies

- Hartley (outbred) guinea pigs can also be used, but require more adaptation of the virus
  - Support for adapting strains of LASV to Hartley guinea pigs for distribution to investigators (e.g. BEI resources)
Guinea pig adapted LASV

4-8 passes in outbred Hartley guinea pigs

Slide courtesy Dave Safronetz, Public Health Canada
Animal Models
research prioritization considerations

Cynomolgus macaques most commonly used NHP model
- Full GLP natural history study to characterize disease course
- To compare vaccines, a well-characterized virus stock should be available to all investigators
- Determine relevant challenge dose (1,000 pfu is standard, but other doses not compared)
- Fully characterize chronic disease course in NHP to include deafness

Rhesus macaques show lower incidence of disease
- Explore as a model for sequelae in survivors?

Marmosets are a good disease model, but are fragile and difficult to work with
Human or rodent isolates?
  - Most studies conducted with Josiah (human)
  - Human: severe vs. mild disease?

Challenge dose
  - TCID50’s vs. PFU/FFU?
  - high titer or low titer?

Route of inoculation:
  - s.c. vs. i.m. vs. mucosal/resp.?

Uniform lethality or not?

Standardized euthanasia criteria

Disease parameters to be measured
  - Telemetry?
  - Viremia?
Terminal Lassa infection in NHPs

- Increased transaminases (ALT, AST)
- Increased ALP, GGT, BUN,
- Decreased albumin & total protein
- Increased PT, aPTT, TT
- Decreased Fibrinogen, Protein S and C activities
- Viral titers equivalent in all tissues analyzed \((10^3 \text{ to } 10^7 \text{ TCID}_{50}\text{'s} / \text{g})\)
- IHC revealed similar patterns of infection
- Histopathological changes similar in most organs

Slide courtesy Dave Safronetz, Public Health Canada
Survivors of experimental challenge
Viremia and Neutralizing Antibodies

Green = survivors
Red = non-survivors

- Survivors (green) were viremic
- Neutralizing antibodies (measured by PRNT) correlated with virus clearance

Hearing Loss in Survivors of Lassa virus Infection

Survivors (green) were chronically ill with symptoms including:

- hunched posture
- reduced appetite
- tremors
- ataxia

All 3 survivors displayed hearing loss by subjective measures; e.g.

- Response to startling sounds
- Tuning fork tests

Pathological Evidence of Autoimmune Vasculitis in LASV Survivors

A. Thickening of hepatic vessels
B. Coronary vessels exhibiting incremental vascular dilation and stenosis (“string of beads”)
C. Multiple foci of interstitial testicular hemorrhage
D. A white fibrous nodular pancreatic mass consistent with previous human autoimmune vasculitis diseases
Circulating immune complexes

Serological Evidence of Autoimmune Vasculitis

Survivors, but not non-survivors, had CIC and ANCA lasting until the end of the study

C-reactive protein is a measure of an ongoing, generalized pro-inflammatory response.

All 3 surviving NHP displayed high levels of C-reactive protein even after viral clearance.

LASV Persistence in Survivors at 45 days post-infection

*In-Situ* Hybridization using an L segment (polymerase) nucleic acid probe

<table>
<thead>
<tr>
<th>Magnification</th>
<th>Survivors</th>
<th>Uninfected</th>
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<tr>
<td>high</td>
<td>low</td>
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**Brain**

**Heart**

**Kidney**

**Liver**

LASV RNA *(red)* was detected in arteries with perivascular lesions in brain, heart, kidney and liver.
Persistent Lassa virus antigen in the arteries with perivascular lesions in the brain

Green = LASV antigen  
Blue = DAPI

Red = endothelial cell marker  
Red = Smooth muscle cell marker

Lassa virus appears to persist in vascular lesions in smooth muscle cells
LASV-Associated Deafness

Conclusions

- Lassa virus infection of cynomolgus macaques causes an acute biphasic disease resulting in death 12-18 days post-infection or survival with chronic, neurological effects.

- Acute sensorineural hearing loss is a consequence of infection in approximately 30% of human survivors, and in our study, we show that a similar proportion of infected cynos also develop deafness, as confirmed by subjective tests and BAERCOM analysis.

- Distribution of severe tissue pathology and deafness in the surviving cynos is most likely due to a systemic autoimmune response that resembles autoimmune-associated deafness in humans.

Auditory waveform is recorded by device.
There is strong evidence for Lassa virus persistence in a variety of tissues after recovery from acute disease.

- Smooth muscle cells surrounding vascular tissue appear to be a target for persistent infection.
- Viral persistence could play a role in chronic effects in observed in survivors.
Research Prioritization
Challenges

- **Characterize virus strains**
  - Process for making gene sequences and isolates readily available to all investigators
  - Process for selecting assays, developing reagents, distributing to all labs

- **Develop animal models**
  - Immune markers demonstrating vaccine efficacy may differ across vaccine platforms
  - For each platform identify relative role of LASV specific antibodies and markers of cell-mediated immunity
  - Animal modeling should also include studies on cross protection, vaccine safety, protection from deafness and neurological deficit