Animal models for Lassa fever

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Outline

• Animal rule
• Caveats / limitations
• Challenge viruses
• Disease models
  – Mice
  – Hartley GPs
  – Inbred GPs
  – NHPs
• Mastomys natalensis
• Future considerations
Animal Rule

• Challenge pathogen should
  – be an etiological agent of human disease
  – have similar pathogenic determents in human disease
  – result in disease following inoculation via similar route as human exposure
  – be reliably quantifiable and reproducibly cause disease

• Host animal should
  – be immunocompetent, outbred
  – susceptible to disease following realistic challenge dose
  – manifest illness/disease which mimics human condition

• Disease condition should
  – be pathophysiologically comparable to human disease with respect to:
    • Time to disease onset
    • Time course of disease progression
    • Disease manifestations including signs, severity, lab parameters, histopathology, organ involvement, morbidity and outcome.
Caveats / Limitations

• Predictive value of each model uncertain
  – Mice vs. GPs vs. NHPs (Marmosets vs. Rhesus vs. Cynos)
  – Value of surrogate models?

• Limitations of working in a BSL4 environment
  – Safety, equipment, personnel, etc.

• Group sizes
  – Difficult to achieve statistical significance (NHPs)
  – Sex/age related differences in disease progression?

• Model severe/lethal disease
  – Little work on mild/moderate clinical manifestations
  – Long term effects??
Challenge viruses

• Human or rodent isolates?
  – Most studies conducted with Josiah (human)
  – Human: severe vs. mild disease?

• Challenge dose: high titer or low titer?
  – TCID50’s vs. PFU/FFU?

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

• Uniform lethality or not?
LF Disease Models

- Mice
- Hartley Guinea pigs
- Inbred Guinea pigs
- NHPs
Guinea pig adapted LASV

![Graphs showing survival of WT LASV and GPA-LASV over days post-infection.]

- **Survival (%)** vs **Days p.i.**
  - **WT LASV**: Green line
  - **GPA-LASV**: Red line
  - *****p < 0.001**

- **Survival (%)** vs **Days p.i.**
  - Different virus doses (10,000 TCID50, 1,000 TCID50, 100 TCID50, 10/1/0.1 TCID50) shown with different colors.
Differing clinical presentations

Strain 13 Guinea Pigs (n = 7)
Challenge dose: $10^4$ TCID$_{50}$'s (i.p.)

Cynomolgus macaque (n = 3)
Challenge dose: $10^4$ TCID$_{50}$'s (i.m.)
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 to 10^7 TCID_{50}’s / g)
- IHC revealed similar patterns of infection
- Histopathological changes similar in most organs

Conjunctiva
Tonsils
Oro/nasopharynx
Nasal mucosa
Trachea
R / L Bronchus
R/ L Lung (upper middle lower)
Lymph nodes (bronchial, mandibular, axillary, mesenteric, inguinal)
Heart
Liver
Spleen
Kidney
Adrenal gland
Pancreas
Jejunum
Colon transversum
Brain (frontal, cerebellum, stem)
Cervical spinal cord
Pituitary gland
Salivary gland
Urinary bladder
Femoral bone marrow
Increased lung pathology in Soromba-R infected NHPs

- Pulmonary lesions of varying severity
  - Soromba-R: Lungs heavy, wet. 50-100% of all lobes affected
  - Josiah / Z-132: 25% of all lung lobes discolored
- Mild to severe interstitial pneumonia
  - Soromba-R = severity score 4
  - Josiah / Z-132 = severity score 1.7

Could an atypical clinical presentation of Lassa infection in the “connecting” countries be responsible for under reporting of Lassa fever in these regions?
**Mastomys natalensis**

- “Multi-mammate rat”
- Life span of 150-180 days
- Relatively sedentary (host range 22 m)
- Co-habitat with humans
- Avg. litter size is 12

**Lassa Infection in rodent host**

- Association of Lassa virus and *M. natalensis* made in 1972
- Horizontal and vertical transmission
- Persistent, lifelong infection (?)
- Asymptomatic
- Shed virus in saliva & urine (up to $10^4$ virus particles /mL)
- Easily isolated from blood/tissues
- Prevalence varies; 5-45%
Lassa virus infection in *M. natalensis*

Three infection patterns:

- **Seronegative / RT-PCR positive**
  - Represent recently infected animals
  - Marker for epizootic transmission of virus
  - Considered high risk for transmitting virus to humans

- **Seropositive / RT-PCR positive**
  - Infection of unknown (but extended) duration
  - Chronically infected ?
  - Risk of to humans ?

- **Seropositive / RT-PCR negative (adults)**
  - Cleared virus (from tissues we analyze) ?
  - LASV latency ?
  - Represent a viable threat to humans / naïve rodents ?
Establishing a lab colony of M. nats

- Founder stock from Doneguebougou
- Dusted, treated with Ivermectin
- Negative for LASV (OW arenas), hantaviruses

Long-term goal: Understanding Lassa virus infection / transmission in the natural host

- Malian export permit
- Vet. health certificate
- IATA transport regs.
- CDC & MT exotic animal permits
- US F&W inspection
- Customs
Results: so far...

- Highly susceptible to LASV infection via multiple routes (i.p., i.m., s.c., i.n., oral)
- ID_{50} < 10 TCID_{50}'s (i.m., s.c. routes)
- Systemic infection (>10 tissues tested), no pathology
- No differences associated with sex
- Self limiting infection, cleared between 30 and 45 dpi (Adults)
- Malian M. nats are susceptible to infection with all LASVs tested to date
- Readily transmissible via direct contact or contaminated fomites
To do:

- Repeat studies in juvenile M. nats
- Assess vertical transmission
- Test disseminating vaccines
Vaccine work to be considered

- Protection against modern Nigerian isolates
- Safety testing
- Efficacy during pregnancy
- Correlates of protection
- Phase I/II trials