Development of a Non-Human Primate Model of Inhalational Tularemia

FDA Workshop on "Current State and Further Development of Animal Models of Serious Infections Caused by *Acinetobacter baumannii and Pseudomonas aeruginosa*"

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Tularemia

- *F. tularensis subspecies tularensis*-type A strains
- Clinical presentation in humans: glandular, ulceroglandular, oculoglandular, oropharyngeal, pneumonic, typhoidal forms
- Primary pneumonic form after inhalational exposure is the most deadly form

NHP Model Development-Inhalational Tularemia

- No new drugs or vaccines currently under regulatory review, focus is on Animal Model Development and Qualification
- *F. tularensis* subspecies *tularensis*, strain SCHU S4
- Cynomolgus macaques:
  - Historical data in NHPs available from studies conducted in 1940s-1960s
  - Readily available, robust species
  - Sourced from mainland Southeast Asia (not Mauritius)
  - All monkeys screened for pre-existing humoral and cell-mediated immunity to *F. tularensis* prior to assignment to study

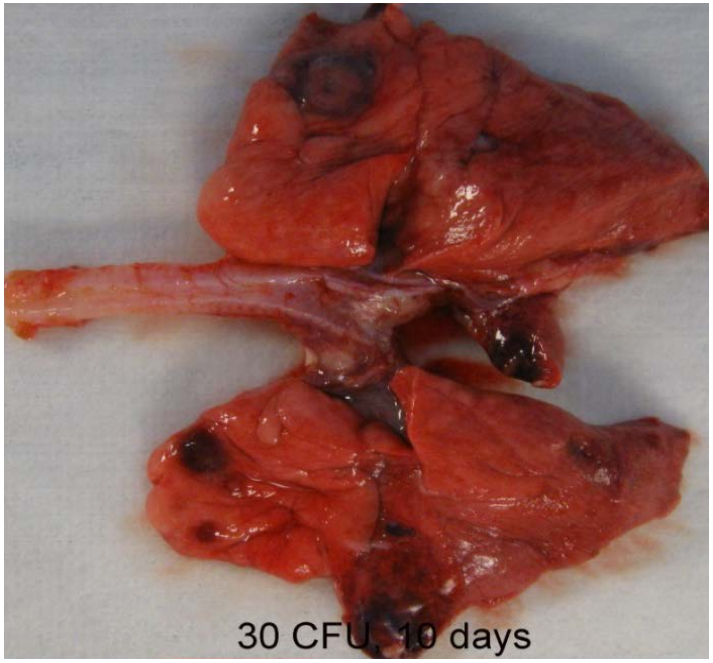
NHP Model Development-Inhalational Tularemia

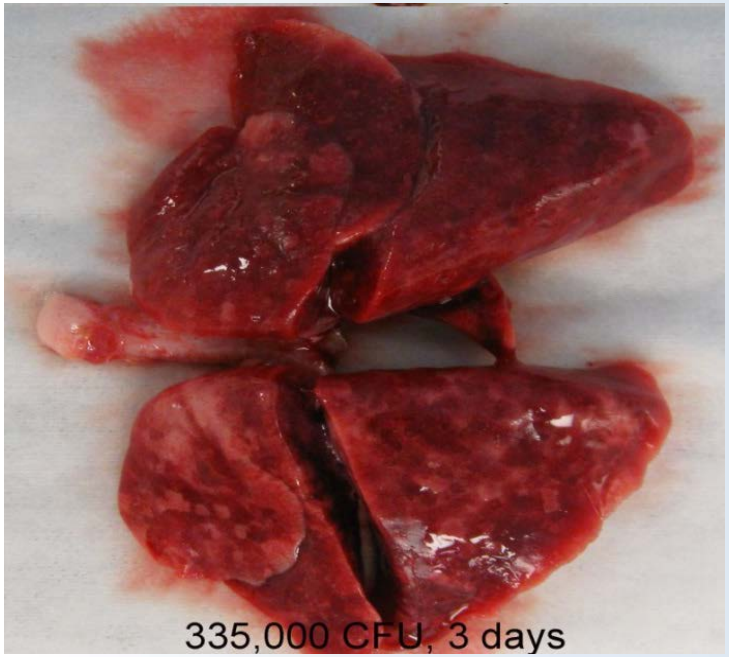
- Approach:
  - Establish LD 50 after head only inhalation exposure to aerosolized SCHU S4
    - Clinical signs, survival, hematology, terminal pathology and microbiology
  - Characterize natural history of disease
    - Telemetry-heart rate, respiratory rate, core body temperature for up to 3 weeks post-challenge
    - Blood for clinical pathology and microbial culture over time in each animal, terminal pathology and culture
    - Serial euthanasia-clinical and anatomic pathology, and microbiology of blood and organs at pre-determined euthanasia time points

LD 50 Study

- 28 male/female cynomolgus macaques, 2-3 yrs of age
- Presented doses from 1.25 to 1.25X10^6 CFU, ~1-3 micron particles; death between day 3 and day 46
- Bacteremia by ~ 3 days post-challenge
- LD 50 < 10 CFU
- Clinical presentation, disease course, time to fever onset and death were dependent upon challenge dose
- Highest doses-rapid death (3 days) with severe bronchopneumonia; clinical signs primarily respiratory
- Lowest doses-survive up to six weeks, with death from disseminated disease; clinical signs primarily anorexia, weight loss, generalized malaise
- Pleuritis; pyogranulomatous to necrotizing lesions in lungs and other organs; variable chronicity; similar to humans

LD50 study: Gross appearance of lungs varied with dose and time to death





Natural History Studies

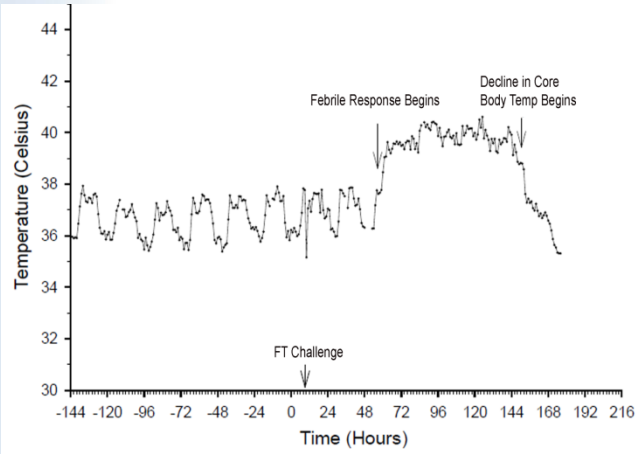
- Challenge dose target-1000 CFU
- Challenge dose rationale:
  - Reproducible challenge dose; reproducible disease manifestations and death; survival long enough to be able to evaluate and compare vaccine or therapeutic efficacy
  - Compared efficacy of new vaccine candidates to Live Vaccine Strain (historical data for comparison)

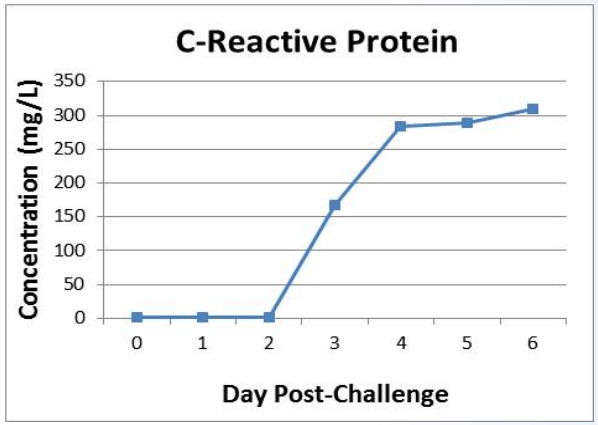
Natural History Studies: Telemetry

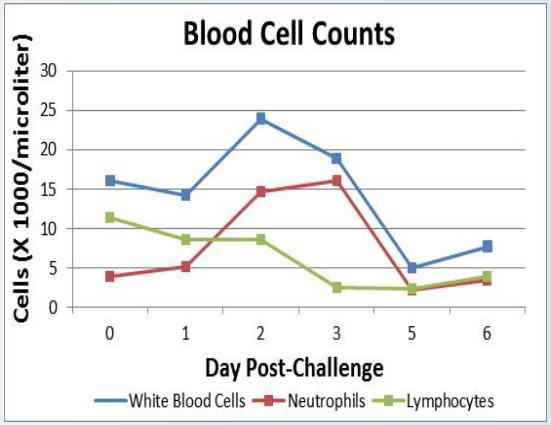
- 12 NHPs, measured core body temp, respiratory rate, heart rate
- Loss of diurnal variation of core body temp, and fever onset between 2-3 days; showed decrease in core body temperature near death (later useful as euthanasia criteria)
- Increases in heart and respiratory rate
- Fever onset useful in later antibiotic efficacy studies as trigger to treat criteria

Natural History Studies: Telemetry

Number of NHPs	Presented Dose (CFU) Mean (± std dev)	Time to Fever (hrs) Mean (± std dev)	Time from Fever to Death (hrs) Mean (± std dev)
N=1	119	60	Study termination
N=11	238 (±162)	47.1 (±3.6)	113.1 (±14.2)







Bacteremia 1st detected on Day 4

Natural History Studies: Serial Pathology

- 16 NHPs, M/F, euthanized 4 each on days 2,4,5,6
- Portal of entry-multiple mucosal surfaces, not just lungs, with some secondary bacterial infections in the nasal cavity
- Local inflammation at site of deposition, followed by inflammation of lymphatic vessels, and draining lymph nodes, and ultimately hematogenous dissemination to macrophage-rich tissues
- Increased WBCs (neutrophils/monocytes) and activation of acute phase response (CRP) between 48 and 72 hrs; increased liver enzymes terminally

NHP Model Development-Inhalational Tularemia

- Challenges/Lessons Learned:
  - Aerosol challenge
    - Well-characterized starting material, *in vitro* growth conditions, and aerosol generation conditions
    - Presented dose is calculated, based on plethysmography-amount deposited and location of deposition vary with breathing rate and depth
    - Particle size influences site of deposition and LD 50
    - Head only challenge allows organism entry from nasal cavity, conjunctiva, oropharynx as well as tracheobronchial tree and deep lung

NHP Model Development-Inhalational Tularemia

- Challenges/Lessons Learned:
  - Telemetry
    - Shallow breathing in NHPs with pleuritis may preclude measurement of respiratory rate
    - SQ temperature chips do not detect fever onset very well
    - HR and RR impacted by human activity in room
  - Prioritize sampling
    - Be cautious that experimental procedures don't impact disease course-(e.g. Diehl guidelines for blood collection are for healthy animals)
  - Establishment of endpoints is critical
    - Use precise terminology (no "moribund euthanasia") and seek input from clinical veterinarian
    - Be consistent in following euthanasia criteria

NHP Model Development-Inhalational Tularemia

- Challenges/Lessons Learned:
  - Collect tissue widely for histopathology-if you don't look for lesions you likely won't find them
    - Safety of test article may also be evaluated in disease model
    - Multiple portals of entry identified, which explained variations in clinical presentation, disease course, time to fever/death, and terminal pathology with challenge dose
    - NHPs are not pristine, inbred animals and background lesions/infections may impact outcomes through non-specific immune stimulation
      - Nosocomial *Serratia* infection in anthrax studies
      - Possible influence of *T. cruzi* infection on *F. tularensis* studies

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