Animal Models for *Streptococcus pneumoniae*

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Major Issues

- Host Selection
- Route of Inoculation
- Strain Selection
- Clinical Endpoints
- Controls
Host Species

- Rodents
  - Mice
  - Rats (advantages?)
  - Chinchillas (otitis media)
- Rabbits (advantages?)
- Primates
  - Non-Human (limited experience)
  - Human

Do we need a small animal for initial testing followed by further testing in primates?
Mouse Models

Advantages
numerous, cheap
colonization and disease
may resemble natural host
host genetics and other tools

Disadvantages
susceptibility varies by ‘type’
Animal Specifics

- Strain - is there an advantage of one strain?
- Age?
- Sex?
- Inoculation Route
  - IN + / - Anesthesia
  - IP
  - IV
Targeting Carriage

- Colonization is the first step in all forms of pneumococcal disease
- A highly effective product would need to induce herd immunity through reduction in colonization
Pneumococcal Strains

• Need for Multiple ‘Diverse’ Strains
  >90 CPS types
  10-20% strain to strain genomic diversity
• Recent Animal Passage
• Intrastrain Variability - Colonizing v. Invasive Phenotypes
Most clinical isolates phase vary between opaque (O) and transparent (T) colony forms that differ in quantity of capsular polysaccharide.
Pneumococcal Variants Expressing Decreased Amounts of CPS are Selected for in the Human Nasopharynx

Source: Drs. S. Gordon and H. Epino from the Queen Elizabeth Hospital, Blantyre, Malawi
Clinical Endpoints

- Reduction in Colonization
- Protection from Disease (Otitis Media, Pneumonia, Bacteremia/Sepsis)
- Correlates of Protection
  - Opsonophagocytosis Assays
  - Antibody
  - Xenogen Detection System

Francis et al, IAI 69:3350. 2001
Antibody Issues

- Is Protection Ab mediated?
- Amount Needed
- Class and Subclass
- Passive v. Active Protection
- Heterologous Source of Ab
- Issues related to IgA and mucosal protection—IgA1 Protease
- Confounding factor of ‘natural’ Ab
Pneumococcal colonization in a murine model: Role of antibody in clearance of *S. pneumoniae* is limited.

Vaccine Control

- Capsular Polysaccharide (Pneumovax)

- CPS conjugated to CRM$_{197}$ (Prevnar)
Model of Experimental Pneumococcal Carriage in Humans

- Performed at Baylor Medical College (Dr. T. Cate)
- 14 healthy adults inoculated with penicillin-sensitive type 23F clinical isolate (also 8 volunteers in a separate study with a type 6B clinical isolate)
- $10^3-4$ CFU inoculated into the nares
- Serum, nasal washes, throat and nasal cultures collected prior to and every 2 weeks after inoculation until no longer colonized for 4 weeks

RESULTS
- 6 Colonized subjects - (27-122 days) asymptomatic
- 8 Uncolonized subjects
Safety Issues

-In healthy individuals asymptomatic carriage is common and disease rare
-When disease occurs there is a stepwise progression of readily apparent symptoms and available treatment

**Inclusion Criteria**

- Age >18<40yrs
- HIV seronegative
- Nonpregnant, nonsmoking,<3 ETOH beverages/day
- Intact spleen
- No pneumococcal vaccination
- No penicillin allergy
- No history of chronic disease or resp. infections
- No close contact at increased risk
- Prescreened for pneumococcal colonization
- Informed consent
Evidence that experimental colonization resembles natural carriage:

- Lack of apparent inflammation
- Low inoculum required
- Duration of colonization
- Individual susceptibility varies and correlates with levels of specific antibody

7 of 8 uncolonized subjects had pre-existing PspA$_{31-189}$-specific IgG compared to only 2 of 6 colonized subjects by ELISA (titer > 1000)

Subject (FS#)

- Pre-inoculation
- Last bleed
Pneumococcal colonization in murine model

- P1121, clinical 23F strain that colonizes without causing disease

10^7 CFU in 10 ul PBS
P1121 intranasally
w/o anesthesia

1. Nasal wash
200 ul PBS
2. Cardiac puncture for serum

Serial dilutions
nasal washes on TS agar plates with neomycin
Vaccine Development:

“A Long Road Where the Obstacles Always Appear to be the Most Apparent Feature Ahead”

Maurice Hilleman
**Immunoglobulin A1 hinge region cleavage sites**

S. pneumoniae expresses an IgA1 protease that cleaves the hinge region of human IgA1 leaving the Fab antigen binding fragment without an associated Fc effector-mediating fragment.