History of early studies on anthrax

Organism and pathogenesis as it relates to immunity

Early approaches to vaccination: live attenuated & acellular protein vaccines

Current licensed U.S. vaccine
  - human & animal data
  - human dose reduction study

New vaccine efforts
History

Disease of Antiquity

1877 - The first disease for which a microbial etiology was determined (Koch)

1881 - Live vaccine developed (Pasteur)
Organism

• Gram-positive, sporeforming, non-motile bacillus

• Virulence factors:
  – Polyglutamic acid capsule
  – Lethal toxin
  – Edema toxin
Variant Strains of *Bacillus anthracis*

Plasmid pX01 (Toxin Proteins)
Plasmid pX02 (Poly D-Glutamic Acid Capsule)
Chromosome

Virulent (Vollum, Ames)
Growth at 42°-43°C

Avirulent (Vollum, Ames)
(not protective)

Avirulent (Sterne, STI)
(live vaccine)
Growth at 42°-43°C

Avirulent (Sterne)

EF (I)
PA (II)
LF (III)

Novobiocin
Anthrax Toxin Components

*Activities, Properties, Nomenclature*

- **Edema Factor (EF)**
  - MW 89,000
  - Adenylate Cyclase
  - Edema in Skin (Rabbits, Guinea Pigs)
  - Increased Cyclic AMP

- **Protective Antigen (PA)**
  - MW 83,000
  - Lethal Factor (LF)
  - MW 90,000
  - Metalloprotease
  - Lethality (Rats, Mice, etc.)
  - Macrophage Lysis

- **Lethal Factor (LF)**
  - MW 90,000
  - Metalloprotease
  - Lethality (Rats, Mice, etc.)
  - Macrophage Lysis
Pathogenesis

- Spore enters skin, GI tract, or lung
- Germinates in macrophage locally or is transported to regional lymph nodes
- Local production of toxins leads to edema and necrosis
- Spreads from node with bacteremia and toxemia
Initial Approaches to Anthrax Vaccines

• Live attenuated strains
  Pasteur & Greenfield

• Acellular *in vivo* expressed antigens (aggressins)
  Bail
Vaccines against Anthrax

Current U.S. Vaccine

Culture supernatant of attenuated non-encapsulated strain V770 NP-1 adsorbed to aluminum hydroxide, made by Bioport (AVA). Composed primarily of protective antigen (PA)

Schedule is 0.5 ml s.c. at 0, 2, 4 weeks followed by doses at 6, 12, and 18 months with subsequent yearly boosters

U.K. chemical vaccine similar to U.S. vaccine

Live attenuated vaccine (STI) used in Former Soviet Union
Evidence For Efficacy Of AVA

**Human:**

- A similar vaccine, which was a less potent precursor of AVA, showed 93% efficacy against anthrax when tested in a placebo-controlled, single blind study in wool mill workers
  - 1 cutaneous case in vaccinated group vs 13 cutaneous and 2 inhalational cases in placebo group
  - There were insufficient numbers of cases of inhalational anthrax, when analyzed separately, to show statistically significant protection
  - However, all 5 cases (2 placebos and 3 non-participants) were in non-vaccinated individuals, suggesting the vaccine was protective
- From 1962 to 1974, “Twenty-seven cases were identified [by the CDC]. Three cases were not mill employees, but worked in or near mills; none of these cases were vaccinated. Twenty-four cases were mill employees; three were partially immunized (one with 1 dose, two with 2 doses); the remainder (89 percent) being unvaccinated. Therefore, no cases have occurred in fully vaccinated subjects while the risk of infection has continued. These observations lend further support to the effectiveness of this product.”
Efficacy of AVA against Inhalational Anthrax

**GUINEA PIGS**

AVA: 9/39 survivors (23%)
Controls: 0/36 survivors

**RABBITS**

AVA: 94/97 survivors (97%)
Controls: 0/88 survivors

**NON-HUMAN PRIMATES**

AVA: 62/65 survivors (95%)
Controls: 0/18 survivors
# Efficacy of AVA in Guinea Pigs

<table>
<thead>
<tr>
<th>Strain/Origin</th>
<th>Surv/Total</th>
<th>(%)</th>
<th>vrrA&lt;sup&gt;1&lt;/sup&gt;</th>
<th>MLVA&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Strain &amp; Origin</th>
<th>Surv/Total</th>
<th>(%)</th>
<th>vrrA&lt;sup&gt;1&lt;/sup&gt;</th>
<th>MLVA&lt;sup&gt;2&lt;/sup&gt;</th>
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<tbody>
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<td>A SIL K7978/ Namibia</td>
<td>1/16 (6)</td>
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<tr>
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<td>3</td>
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<td>Ames/ USA</td>
<td>2/16 (13)</td>
<td></td>
<td>4</td>
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<tr>
<td>A SIL K5926/ India</td>
<td>2/16(13)</td>
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<td>5</td>
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<td>5</td>
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<td>A SIL K1938/ Indonesia</td>
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<td>A3</td>
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<td>5</td>
<td>A4</td>
<td>BA 1018/ South Africa</td>
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<tr>
<td><strong>Other Isolates</strong></td>
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<td><strong>Other Isolates</strong></td>
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<tr>
<td>33/ South Africa</td>
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<td>B1</td>
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<td>B1</td>
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<td>6</td>
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<td>2</td>
<td>B1</td>
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<td></td>
<td>4</td>
<td>A4</td>
<td>A SIL K6093/ Croatia</td>
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<td>BA 1033/ South Africa</td>
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<td>8/16 (50)</td>
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<tr>
<td>A SIL K2087/ USA</td>
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<td></td>
<td>4</td>
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<td>8/16 (50)</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>A SIL K7282/ Germany</td>
<td>9/16 (56)</td>
<td></td>
<td>3</td>
<td></td>
<td>A SIL K7282/ Germany</td>
<td>9/16 (56)</td>
<td></td>
<td>4</td>
<td>A1</td>
</tr>
<tr>
<td>BA 1002/ Vollum 1B</td>
<td>9/16 (56)</td>
<td></td>
<td>2</td>
<td></td>
<td>BA 1007/ USA</td>
<td>9/16 (56)</td>
<td></td>
<td>4</td>
<td>A1</td>
</tr>
<tr>
<td>A SIL K6286/ Canada</td>
<td>12/16 (76)</td>
<td></td>
<td>4</td>
<td></td>
<td>A SIL K6286/ Canada</td>
<td>12/16 (76)</td>
<td></td>
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<tr>
<td>BA 0018/ Canada</td>
<td>16/16 (100)</td>
<td></td>
<td>3</td>
<td>A1</td>
<td>BA 0018/ Canada</td>
<td>16/16 (100)</td>
<td></td>
<td>3</td>
<td>A1</td>
</tr>
</tbody>
</table>

<sup>1</sup> Variable number tandem repeat (VNTR) category for vrrA

<sup>2</sup> Multiple-locus VNTR analysis genotype
Summary and Conclusions

• Guinea pigs vary in the effectiveness of AVA when tested against a geographically diverse group of *B. anthracis* isolates

• There is no relationship between virulence in AVA vaccinated guinea pigs and current genotype analysis of *B. anthracis* isolates

• Both rabbits and rhesus macaques vaccinated with AVA are resistant to challenge with isolates that overcome AVA vaccination in the guinea pig
In vitro Correlate of Immunity Study Design

• Species: NZW Rabbits, both sexes
• Route: Intramuscular
• Schedule: Days 0, 28
• Immunization: Varying doses (Human - 1:256)
  – Sera: Day 42
  – Assays: Anti-PA ELISA / Toxin Neutralizing Ab
• Challenge: Aerosol, Day 70
  – Spores: Ames Strain
Confirmation of an *in vitro* Correlate of Immunity in a Rabbit Model for Inhalational Anthrax

*Significance of odds of survival*
Correlates of Protection

- Antibody to PA (ELISA) and toxin neutralization correlate with immunity induced by AVA in the rabbit.
- Antibody to PA (ELISA) correlates with immunity induced by live attenuated vaccine in the guinea pig.
- Toxin neutralizing antibody is a better correlate of immunity than ELISA in the guinea pig vaccinated with PA.
Mechanism of AVA-induced Protection

- Induction of antibodies neutralizing lethal toxin
- Induction of antibodies inhibiting spore germination
- Induction of antibodies enhancing spore phagocytosis and increasing rate of killing
## Anthrax IgG PA Elisa Titers of Human Sera Obtained 47-49 Days after First Dose of Anthrax (USAMRIID Special Immunizations Program)

<table>
<thead>
<tr>
<th>Interval between First Two Doses (wks)</th>
<th>No. of People</th>
<th>% with Titer &gt;1:100</th>
<th>Geometric Mean Titer</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>22</td>
<td>95%</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>100%</td>
<td>1225</td>
<td>0.0090</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>100%</td>
<td>1860</td>
<td>0.0015</td>
</tr>
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</table>

a Analysis of variance with pairwise tests of intervals.
Human Anthrax Vaccine Studies

• Overall objectives
  – Reduce total AVA doses from 6 to 4 with annual boosters
  – Optimize route
Goals of Study 1

• Determine the best two dose schedule for anthrax vaccine administration

• Determine the better route (IM vs SQ) of anthrax vaccine administration

• Collect safety data
### Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination Schedule</th>
<th>SQ</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 week</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0-2</td>
<td>0-2 weeks</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0-4</td>
<td>0-4 weeks</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>0-2-4</td>
<td>0-2-4 weeks, 6-12-18 months</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

- Prospective study in which volunteers were randomized to one of seven groups (six study groups or one control group)
- Annual report submitted to FDA October 1997 (BB-IND 6847, Amendment No. 005)
Comparison of Anti-PA IgG Concentrations: Standard Vaccination Schedule vs Weeks 0-4

Bars represent 1 standard error of geometric mean
Anthrax Vaccine Study 1 Summary

• GM Anti-PA IgG concentration at peak is not different for the standard schedule and 0-4 IM or 0-4 SQ reduced schedules

• Rate of decline of antibody concentration from week 7 to 24 is not different for the three groups

• Seroconversion rates for the three groups range from 95-100%

• Females have significantly higher local reaction rates compared to males when AVA is administered via the SQ route.

• Local reactions are none to minimal with the IM route compared to the SQ route (SQ nodules, redness, induration)

• Increasing time between the first two doses from 2 to 4 weeks SQ significantly reduces the local inflammatory reaction

• A larger randomized, placebo-controlled, double-blinded study is required to confirm these results and for FDA to allow supplement to licensure

• Such a study is being conducted by CDC in collaboration with DoD and NIH
Approaches To New Vaccines

**PROTEIN VACCINES** (Better characterization, fewer doses)
- rPA (various expression systems)
- PA (mutants) +/- LF/EF (mutants)
- Other adjuvants and delivery systems

**LIVE ATTENUATED VACCINES**
- *B. anthracis* (Sterne, Aro-, PA+/- LF/EF mutants)
- *B. subtilis* (PA)
- *Salmonella* (PA)
- Vaccinia (PA)
- *Lactobacillus casei* (PA)
- *Francisella tularensis* LVS (PA)

**OTHER**
- DNA
- VEE replicon
- Plants for oral delivery

**IDENTIFICATION OF NEW IMMUNOGENS**
- Spore antigens
Current Status of Research on Recombinant PA (rPA) Candidate Vaccine for Anthrax

• Developed non-spore forming *Bacillus anthracis* strain to produce rPA
  – (will not require a dedicated manufacturing facility)

• Determined production and purification methods for rPA under GMP conditions

• Demonstrated protection by rPA vaccine against aerosol challenge in rabbit and non-human primate models

• Identified alternative production method and source for rPA produced in *E. coli*

• Phase 1 clinical trial designed
Efficacy of rPA against Inhalational Anthrax

<table>
<thead>
<tr>
<th></th>
<th>GUINEA PIGS</th>
<th>RABBITS</th>
<th>NON-HUMAN PRIMATES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rPA 21/60 survivors (35%)</td>
<td>rPA 25/30 survivors (83%)</td>
<td>rPA 64/68 survivors (94%)</td>
</tr>
<tr>
<td></td>
<td>Controls 0/10 survivors</td>
<td>Controls 0/10 survivors</td>
<td>Controls 0/11 survivors</td>
</tr>
</tbody>
</table>
rPA Phase 1 Clinical Trial Study Design

• Outpatient, Randomized
• Dose escalation
  – 10 study volunteers per cohort
    • group 1 (5 µg + alhydrogel)
    • group 2a (25 µg) + alhydrogel)
    • Group 2b (25 µg)
    • group 3 (50 µg + alhydrogel)
    • group 4a (100 µg + alhydrogel)
    • Group 4b (100 µg)
      * Males and non-pregnant females
      * Ages 18 years and older

• Criteria for Dose Escalation
  – No evidence for safety concerns with administered doses
  – Clinically significant safety issues reviewed by Safety and Data Committee
ACKNOWLEDGMENTS

AVA HUMAN STUDIES


AVA EFFICACY AGAINST GEOGRAPHICALLY DIVERSE STRAINS

P. Fellows, M. Linscott, B. Ivins, L. Pitt, C. Rossi, P. Gibbs

SPECIES RESPONSE TO AVA

S. Little et al.

IN VITRO ANTI-SPORE EFFECTS OF ANTIBODIES TO PA

S. Welkos, S. Weeks, S. Little, I. Mendelson, D. Fritz, P. Fellows

AEROSOL STUDIES WITH AVA AND rPA

L. Pitt, B. Ivins, P. Fellows, S. Little, S. Welkos, J. Estep, R. Hunt
ACKNOWLEDGMENTS

ADJUVANTS


DNA VACCINES

C. Schmaljohn, A. Ruff, L. VanderZanden, B. Ivins, P. Fellows

VEE REPLICON

J. Lee, P. Pushko, J. Smith, S. Welkos

LIVE VACCINES

J. Barnard, S. Tobery, D. Chabot

rPA ADVANCED DEVELOPMENT