IMMUNIZATIONS AGAINST BIOTERRORISM

Smallpox and Anthrax Vaccines

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OBJECTIVES

- Briefly review the rationale for continued development of smallpox and anthrax vaccines
- Discuss licensed vaccines for prevention of smallpox and anthrax
- Summarize approaches to the development of improved vaccines
• Anthrax and smallpox are ancient diseases that have been used to cause disease in humans + animals.

• Many nations have engaged in biological weapons programs.

• More recently, concerns regarding the use of biological weapons by terrorists have grown.

• Low infectious doses and the potential for aerosolization of these pathogens could result in the exposure of millions of people.

• Improved vaccines are desirable for both.
NEW VACCINES AND REGIMENS Considerations

- Vaccine Characterization
- Safety and Immunogenicity
- Regimens (Pre- and Post-Exposure)
- *Demonstration of Efficacy (Animal Rule)*
- Correlates/Determinants of Protection
- Duration of Protection/Need for Boosters
- Adjuvant Content
- Use in Other Populations (Young, Old, Pregnant, Underlying Medical Conditions)
Bacillus anthracis
From Woolsorters to Mail Sorters

• Gram + spore-forming rod
• Primarily causes diseases of animals
• Inoculated, ingested or inhaled spores are infectious (industrial, agricultural, laboratory & terrorism)
• Vegetative form elaborates plasmid-encoded toxins (pX01) and a \( \gamma \)-linked, poly-D-glutamic acid capsule (pX02; antiphagocytic)
• Continued proliferation results in invasion, toxemia (hemorrhage, necrosis, edema), shock & death
MOLECULAR PATHOGENESIS OF ANTHRAX
Pivotal Role of PA

Lethal Factor (LF)

Protective Antigen (PA)

Binding Subunit

Edema Factor (EF)

Lethal Toxin: Zinc Metalloprotease

Edema Toxin: Adenylate Cyclase

MAPKK cleavage inhibits MAPK pathway
MØ+ endothelial cell apoptosis,
cytokine modulation, hypoxia,
PMN, PLT+ DC dysfunction, toxic shock

ATP→cAMP
Dysregulates water homeostasis (edema), inhibits
lymph prolif, phagocytosis,
oxidative burst, mediates cell
death, cytokine release
Bacillus anthracis
Correlates of Protection

• Immune responses following infection vs. capsule and PA > LF > EF (LF greater in one study: Doganay M et al., 2011)
• Fully effective vaccines contain PA
• PA immunization alone protects in models; passive immunization with anti-PA protects
• Anti-LF contributes to protection
• Anti-PA and toxin neutralizing antibody (TNA) levels correlate with protection
• Other components (may) also contribute (capsule, spore; CD4+ T cells)
ANTHRAX VACCINE DEVELOPMENT

- Live spore vaccines in use since the 19th C.
- 1904: Immunization of animals with edema fluid from cases stimulated protection (Bail)
- 1946: PA produced in vitro (Gladstone)
- 1953: PA yields improved (Belton and Strange)
- 1951-1955: Vaccine developed at Fort Detrick; used to immunize scientific personnel (Wright); immunization regimen= 6 doses SQ over 18 mo with annual boosters
- Efficacy demonstrated in 1,249 goat hair mill workers in the NE US; 1955-1959: 92% protection

ANTHRAX VACCINE ADSORBED (AVA)

Licensed by the US FDA in 1970

- Prepared from a toxigenic, nonencapsulated strain of \textit{B. anthracis (V770-NP1-R)}-BioThrax®
- Cell-free filtrate contains a mixture of cellular products adsorbed to aluminum hydroxide: PA > LF >> EF (if present) and others
- Benzethonium chloride and formaldehyde (preservative + stabilizer) present in the final product
- A similar vaccine is licensed in the UK (Sterne 34F₂); alum-adjuvanted + thimerosal; higher LF/EF; its use reduced the number of cases in wool disinfection stations.
- Vaccines confer significant protection in animals
Anthrax Vaccine Research Program

CDC AVA CLINICAL TRIAL DESIGN

Based on preliminary data from Pittman et al. Vaccine 20:1412, 2002

Healthy adults were randomized to receive AVA or placebo according to one of the schedules outlined above. Safety, reactogenicity and immunogenicity were assessed over 43 months.
CDC AVA CLINICAL TRIAL

Summary of Analyses to Date

- Lower level of injection site reactions when vaccine was administered IM vs. SQ; more reactions in females
- Antibody responses at 7 months similar even if the 2-week dose is dropped; antibody responses in 4-IM group superior to those in other groups at 43 months
- US FDA approved change in route to IM for pre-exposure prophylaxis, & dropping the dose at week 2.
- At 43 months, 4IM superior to other regimens—will less frequent boosting be adequate?

Sabourin C et al., Quinn Q et al. 2010 ASM Biodefense & Emerging Diseases Mtng.
Similar levels of antibody and frequencies of responders at 7 months after IM or SQ immunization with 3 or 4 doses of AVA

AVA
Safety and Reactogenicity

- Injection site reactions common (SQ > IM): Tenderness, pain, redness, swelling, itching; more frequent among women
- Systemic reactions infrequent
- Hypersensitivity reactions on occasion
- Most symptoms resolve within several days
- Persistent subcutaneous nodules may develop at the injection site
- Safety supported by numerous studies and reviews; 1st trimester vaccination resulted in slightly higher rates of birth defects (p=NS when compared to women vaccinated outside of pregnancy)
AVA
Recommendations for Use

- Indications: Persons at high risk of exposure to spores (lab workers, others whose occupation may involve handling infected animals or other contaminated materials); military; first responders
- Contraindications/warnings: Immediate allergic reactions; prior history of anthrax
- Pre-Exposure Regimen: 5 IM doses at day 0, week 4, months 6, 12 and 18 followed by annual boosters For UK vaccine: 3 IM doses 3 weeks apart, a dose at 6 months; then annual booster
- Post-Exposure Regimen (AVA): 3 SQ doses at 0, 2 and 4 weeks with a 60-day course of antibiotic
ANTHRAX VACCINE DEVELOPMENT

Newer Approaches

- Recombinant PA vaccines: Advanced development
  - *E. coli*-based (Brown B et al. PLoS ONE 2010; 5:e13849; SparVax®,
    initially produced by DSTL in the UK)

  Additional antigens: LF, capsule, and spore antigens

- DNA constructs (Keitel W et al. Human Vaccines 2009; 5:536-44)

- Alternative routes of administration (Intranasal, transcutaneous)

- New adjuvants (CpG, MPL, MF59, LT, etc.)

- Live attenuated organisms and vectors

- Spore-based vaccines

See Baillie L. Human Vaccines 2009; 5:806
Variola (Smallpox)

- DNA virus; member of the Orthopox family
- Human pathogen
- Naturally spread by close contact with infected people or contaminated materials
- Causes a systemic cytocidal infection with up to a 30% CFR (greatest killer in history?)
- Once a common and deadly disease, the disease was declared eradicated by WHO in 1980.
SMALLPOX
Correlates of Protection

• Potent immunity for at least 3-5 years after vaccination (protection vs. monkeypox >30 yrs)
• Serum neutralizing antibody critical; cell mediated immune responses important for controlling primary infection (Ab and CMI can persist for decades)
• Major reaction or ‘take’ after vaccination
  • Vesicular or pustular lesion
  • Area of palpable induration or congestion surrounding a central lesion (crust or ulcer)

Moss B. Immunological Reviews 2010; 239:8-26 Rimoin A et al. PNAS 2010;107:16262
Jenner published observations that material from the lesions of cowpox could protect humans from smallpox; vaccination became widespread.

The origin of vaccinia virus is not known. However, several strains were used in the eradication program: NYCBH (US), Lister (UK), Temple of Heaven (China) and EM-63 (USSR) and others.

Military vaccination in US resumed in 2002 for units that will deploy to ‘high risk’ areas.

Desire to stockpile vaccine for civilian populations resulted in a series of clinical trials of old and new vaccines.

‘Inquiry into the Causes and Effects of the Variolae Vaccinae,’ 1798
US SMALLPOX VACCINES; 2001
Are Older Vaccines Potent/Safe?

- **Dryvax (Wyeth); Lyophilized**
  - Derived from NYCBH
  - Produced 1974-81
  - Stored at CDC
  - ~15 million doses

- **Aventis Pasteur Vaccine (SPSV); Liquid**
  - Derived from NYCBH; a.k.a ‘Wetvax’
  - Produced in 1956-57 for the military
  - Stored at -20C in Swiftwater, PA
  - ~70 million doses
SMALLPOX VACCINES
NIAID Dose-Sparing Trials

- Vaccinia-naïve: Doses of $10^{8.1}$, $10^{7.2}$ or $10^{7.0}$ pfu/mL (Dryvax) resulted in takes in 97-99.1%. All doses were reactogenic: rashes, adenopathy, fever headache, nausea, muscle aches, fatigue and chills were noted. Take rates $\leq 70\%$ with doses of $\leq 10^{6.5}$. In non-naïve: 90% & 95% take rates with $10^{7.0}$ & $10^{7.5}$.


- Groups given doses of $10^{6.2}$-$10^{8.2}$ pfu/mL of Dryvax or $10^{6.6}$-$10^{8.1}$ pfu of SPSV had similar take rates and Neut Ab responses, but systemic reactions rates and frequency of missed activities were lower for groups given lower doses of vaccine.

Couch RB et al. J Infect Dis 2007; 195:826
CUTANEOUS LESIONS AFTER SMALLPOX VACCINATION

Satellite lesions

Vesicular

Lymphangitis

Erythema multiforme

Maculo-papular
### RARE ADVERSE EVENTS AFTER IMMUNIZATION (per million vaccinations)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Primary</th>
<th>Revacc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadvertent inoculation</td>
<td>25-529</td>
<td>42</td>
</tr>
<tr>
<td>Generalized vaccinia</td>
<td>23-242</td>
<td>9</td>
</tr>
<tr>
<td>Eczema vaccinatum</td>
<td>10-39</td>
<td>3</td>
</tr>
<tr>
<td>Progressive vaccinia</td>
<td>0.9-1.5</td>
<td>3</td>
</tr>
<tr>
<td>Post-vaccinial encephalitis</td>
<td>3-12</td>
<td>2</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>?</td>
</tr>
</tbody>
</table>
SECOND GENERATION VACCINE

ACAM2000®

- Safety profile of 1st generation vaccines questioned-pool of strains; growth on calf skin
- A single plaque-purified vaccinia virus derived from Dryvax® (NYCBH strain) was grown in Vero cells
- Clinical and immunologic responses are similar to first generation vaccines
- As with Dryvax®, a high rate of myocarditis and pericarditis have been observed (*vaccinia-naïve; rates between 1.3-10.4/1000 reported*).
- Licensed; US FDA (2007), replacing Dryvax®


ACAM1000, ACAM2000 vs. Dryvax
RCD of Serum Neutralizing Antibody Titers

Frey S et al. Vaccine 2009; 27:1637-44
See also Monath T et al. Int J Infect Dis 2004; 852, 531-44
SMALLPOX VACCINE
Use in Non-Emergency Conditions

- Recommended for laboratorians who work with orthopox viruses, and military/public health and health care response team members (15 punctures)
- Contraindications: Allergy to components; pregnancy/planning pregnancy; breastfeeding; certain skin conditions (eczema; atopic dermatitis); weakened immune system; heart condition or ≥ 3 risk factors; certain household contacts
- No absolute contraindications in a post-event situation-vaccinate within 3 days, if possible....

CDC. MMWR 52 (RR04); 1-28, 2003  Breman JG and Henderson DA. NEJM 338:556, 1998
LC16m8
Attenuated Smallpox Vaccine

- LC16m8: Based on Lister strain; attenuated via tissue culture and chorioamniotic membrane passage. Replicates in skin. Developed and licensed in Japan in the 1970s; stockpiled.
- Take rates were comparable among Japanese children vs. Lister and Ikeda; lower injection site, fever and systemic reactions (>10^5 children; 1970s).
- Take rates and seroconversion rates in naive and revaccinated Japanese military personnel were 94.4/90.2% and 86.6/60%, respectively; vaccine was well tolerated. Safe in patients with ‘dry and stable’ atopic dermatitis.

SMALLPOX VACCINES

Second, Third Generation & Beyond

- Tissue Culture-Based, Live Virus Vaccines
  - CCSV & CJ-50300: MRC-5 cells; Elstree-BN
- Attenuated Vaccines
  - Modified Vaccinia virus Ankara (MVA): CEF-passaged
    - Imvamune®: Safe, immunogenic, and reduces reactions to subsequent Dryvax vaccination. For use in immune compromised, etc? Frey S et al. Vaccine 2007; 25:8562
    - Krempelhuber A et al. Vaccine 2010; 28:1209
- Subunit Vaccines; VACV-rPA Merkel T et al. PNAS 2010

SUMMARY

• Effective vaccines for the prevention of anthrax and smallpox are available. However, experience with anthrax vaccine in populations other than healthy adults is limited/absent, and the high rate of adverse events and restrictions on the use of smallpox vaccines are problematic.

• New vaccines/immunization regimens in development may improve safety and expand the indications for use. Poly-and monoclonal antibodies and antimicrobials may be required for optimal control.

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5829a1.htm
Davies E et al. MMWR 2009; 58:797-800
EXTRA SLIDES
rPA: Phase I Clinical Trial in Healthy Adults (*B. anthracis* – based)

rPA: Phase I Clinical Trial in Healthy Adults (*E. coli* - based)

*Brown B et al. PLoS ONE 2010; 5:e13849*

*See also Campbell J et al Hum Vaccines 2007;3:205.*
AVA +/- CpG Adjuvant

From Rynkiewicz D, et al. Presented at ICAAC
DNA Vaccine (PA + LF plasmids + Adjuvant)  
**Percent Responding After 3 Doses**

Keitel W et al. Human Vaccines 2009; 5:536-44

Dose-response relationships for anti-PA and anti-LF observed
ANTHRAX VACCINES: SUMMARY

- Live Cellular Vaccines (Primarily for animals)
  - Sterne type live spores (toxigenic, unencapsulated)
  - Former USSR STI live spores (same as Sterne)
  - Pasteur type (mixed culture, reduced virulence)
- Sterile Acellular Vaccines for Human Use
  - US anthrax vaccine adsorbed (AVA)
  - UK anthrax vaccine precipitated (AVP)
- Others Human Vaccines (in development)
  - Recombinant PA vaccines with adjuvants
  - Live attenuated, DNA, Others [capsule, spores (incl. B. subtilis)]
- Passive Immunization (polyclonal and MoAbs)