Efficacy of rSEB Vaccine and CpG ODN Administered by Inhalation in Monkeys

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Approved for public release, distribution unlimited

Introduction/concept

- Staphylococcal enterotoxins (SEs)
  - family of exotoxins (23-29 kDa) produced *Staphylococcus aureus*
  - categorized as a superantigen
  - stimulate T cells
  - release of cytokines (e.g., interferon-γ, IL-6 and TNF-α)
- Primates/humans very sensitive to SEB due to ↑ MHC class II binding affinities
- MHC polymorphisms within human populations contributes to individual differences in susceptibility to the effects of SEB
  - Inbred mice differ in MHC alleles – results in varying sensitivities to SEB
  - rodents are not very susceptible
- A recombinant SEB vaccine (rSEBv) developed at USAMRIID protected both rodents and nonhuman primates from lethal aerosols of SEB
  - enteric effects observed in vaccinees
  - May stem from lack of mucosal immunity against SEB
  - Both systemic and *mucosal* immunity is thought to be important when protecting against SEB
    A proteosome-toxoid SEB vaccine admin. mucosally to monkeys resulted in 100% protection against SEB aerosol challenge and an overall reduction of enteric effects,
      - this observation was not quantifiable (Lowell et al., 1996).
Proof of concept: Mouse study

two strains of mice used in study

- In addition to efficacy, was imperative to
  - Produce enough vaccine to support aerosol vaccination
  - Characterize mucosal adjuvant
    - Initial toxicity
    - As an inhalable indication
  - Characterize antigen as inhalable
  - Define proportions of inhalable dose
  - Revisit vaccination schedule
  - Define immune response before and after vaccination
    - characterize unusual adverse effects associated with this route of administration (i.e., hypersensitivity)
Vaccine dosing and challenge

- **Vaccination (rSEBv)**
  - 5 µg (aerosol)
  - 20 µg (intramuscular; IM)

- **Adjuvant**
  - 5 µg (CpG; aerosol)
  - 20 µg (CpG; IM)
  - 180 µg (Alum; IM)

- **Combinatory groups**

- **Challenge**
  - SEB whole toxin (7 LD$_{50}$s)
  - aerosol

- **LPS potentiation**
  - 75 µg (IP; BALB/c)
Mouse data: serum IgG

**Figure:**

- **HLA-DQ8**
- **BALB/c**

- Comparative absorbance (450 nm) for different dilutions and treatments:
  - rSEBv (i.m.)
  - rSEBv + CpG (i.m.)
  - rSEBv (aerosol)
  - rSEBv + CpG (aerosol)
  - sham
  - alum

- Data points are indicated for various dilutions (10, 100, 1000, 10000, 100000).
IgA in Saliva

![Bar graph showing IgA levels in saliva with different treatments.](image)

- **rSEB (aero)**
- **rSEB+CpG (aero)**
- **rSEB+CpG (im prime; aero boost)**
- **rSEB (im prime; aero boost)**

Legend:
- DQ8+
- BALB/c

*Note: The graph shows absorbance at 405 nm for different treatments at various boosts.*
Figure 1. Comparative protection of mice against a lethal dose of aerosolized SEB in BALB/c (a) or HLA-DQ8 transgenic (b). Optimization of vaccine schedule (c) indicated four vaccinations provided optimal protection in the BALB/c mouse.
Primate study

• From mouse data
  – established vaccination schedule
  – Expected survival rates

• Specific to NHP study
  – characterize possible reduction in enteric effects
  – unknown adverse events in higher species
  – FDA-approved device for aerosol vaccination¹
    • lower demand on vaccine stocks (higher efficiency of delivery)
    • Produces equivalent aerosol size as nebulizer used in mouse study (MMAD = 1 µm)

¹Provided in collaboration with Aerogen Corporation, Sunnyvale, CA (USAMRIID CRADA)
vaccination schedule

Assessment of response to vaccine administration:

- acute pulmonary toxicity/reactogenicity
- clinical chemistries
- telemetry (temp. & activity)
- antibody development (serological and mucosal)
## Summary of Primate Experiments

### Phase I

**Purpose:** to determine which administration route for the vaccine provides protection against lethality and enteric effects of SEB intoxication.

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination</th>
<th>Administration route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sham</td>
<td>IM</td>
</tr>
<tr>
<td>2</td>
<td>CpG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>aerosol</td>
</tr>
<tr>
<td>3</td>
<td>rSEBv&lt;sup&gt;a&lt;/sup&gt; + CpG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>IM</td>
</tr>
<tr>
<td>4</td>
<td>rSEBv&lt;sup&gt;b&lt;/sup&gt; + CpG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>aerosol</td>
</tr>
</tbody>
</table>

### Phase II

**Purpose:** to determine if adjuvant is necessary when administered by inhalation to provide protection against lethality and enteric effects of aerosolized SEB.

<table>
<thead>
<tr>
<th>Group</th>
<th>Vaccination</th>
<th>Administration route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rSEBv&lt;sup&gt;a&lt;/sup&gt;</td>
<td>IM prime; aerosol boost</td>
</tr>
<tr>
<td>2</td>
<td>rSEBv&lt;sup&gt;a&lt;/sup&gt; + CpG&lt;sup&gt;b&lt;/sup&gt;</td>
<td>IM prime; aerosol boost</td>
</tr>
</tbody>
</table>

<sup>a</sup> 20 μg/vaccination (measured)

<sup>b</sup> 20 μg/vaccination (nominal)
aerosol administration of vaccine

- Delivers a fine particle, low-velocity aerosol*
- Precisely-defined particle size
- Aerosolizes a broad range of formulations

<table>
<thead>
<tr>
<th>Nebulizers</th>
<th>Clinic use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalers</td>
<td>Field Use</td>
</tr>
</tbody>
</table>

*provided via USAMRIID CRADA with Aerogen Corp.
Acute Pulmonary toxicity

Lung lavage

– CpG Oligo 10103 delivered by aerosol. Photos at 100x. (a) preexposure, (b) 24 hours postexposure.
assessed prior to and 24 hours after aerosol administration of vaccine at the primary tracheal bifurcation (figure a) and the left secondary superior and inferior lobar bronchus (figures b, c, d).

**Results of visual observations:**

- no discernable toxicity
- minor trauma from bronchoscope procedure
- pinpointed location of BAL sample
clinical chemistries

![Graphs showing clinical chemistries measurements for different groups.](image-url)
Serum Anti-SEB IgG development in rhesus monkeys one week before aerosol challenge using either rSEB alone, CpG ODN alone, or comixed administered either by injection, inhalation, or combination of routes thereof.
Serum Anti-SEB IgG development in rhesus monkeys one week before aerosol challenge using either rSEB alone, CpG ODN alone, or comixed administered either by injection, inhalation, or combination of routes thereof.
S-IgA development in rhesus monkeys one week before aerosol challenge (+98d). Individual values are presented for the following groups: (a) rSEB+CpG (im), (b) sham (im); (c) rSEB + CpG ODN (aerosol), and (d) CpG ODN (aerosol).
IgA from saliva samples in rhesus monkeys one week before aerosol challenge using either rSEB alone (a) or rSEB + CpG ODN (b) administered by injection at prime vaccination with boosts by aerosol.
Incapacitation: Clinical Scoring

- Clinical signs observation performed 6 times/day post challenge
- Categorical scaling system adapted from SEB clinical signs
  
<table>
<thead>
<tr>
<th></th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>NO EVIDENCE OF SYMPTOMS</td>
</tr>
<tr>
<td>1</td>
<td>PRESENCE OF SYMPTOM IN A MILD OR UNDETERMINED DEGREE</td>
</tr>
<tr>
<td>2</td>
<td>INDICATES A DEGREE NOTABLE FOR REQUIRING THERAPY</td>
</tr>
<tr>
<td>3</td>
<td>SEVERE</td>
</tr>
</tbody>
</table>

- Signs were graded for each animal

<table>
<thead>
<tr>
<th>SIGN</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOMITING/HEAVING</td>
<td>ACTIVE VOMITING OR WRETCHING; DRY HEAVING</td>
</tr>
<tr>
<td>COUGH</td>
<td>ANY SUDDEN EXPPELLING OF AIR FROM THE LUNGS INDICITIVE OF A COUGH</td>
</tr>
<tr>
<td>MALAISE</td>
<td>LACK OF MOVEMENT RELATIVE TO PRE-EXPOSURE LEVEL</td>
</tr>
<tr>
<td>WEAKNESS</td>
<td>DIFFICULTY GRABBING ON TO CAGE; OR ABILITY TO HOLD UP ARMS</td>
</tr>
<tr>
<td>ANOREXIA</td>
<td>COUNT BISCUITS CONSUMED BASED ON CARETAKER FEEDING SCHEDULE</td>
</tr>
<tr>
<td>MUCOUS MEMBRANES</td>
<td>DRYNESS CHANGES</td>
</tr>
<tr>
<td>LABORED BREATHING</td>
<td>ACTIVE EXPIRATION; DIFFICULTY WITH INHALATION</td>
</tr>
</tbody>
</table>
Clinical Outcome Results (Phases I & II)

sum mean of group (n=4) incap. score (6 obs/day)

hrs post exposure

- rSEB+CpG (im)
- rSEB+CpG (aero)
- SHAM
- CpG (aero)
- rSEB+CpG (com)
- rSEB (com)
Telemetry: Physiological Response to SEB challenge

- **rSEB+CpG (im)**
- **rSEB (im prime; aero boost)**
- **sham**
- **rSEB+CpG (aero)**
**primate survival**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vacc. Route</th>
<th>LD50s (mean ± s.d.)</th>
<th>alive/grp</th>
<th>(%)</th>
<th>MTD (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>injection (im)</td>
<td>16.0 ± 2.5</td>
<td>0/4</td>
<td>(0)</td>
<td>43.0 ± 12.9</td>
</tr>
<tr>
<td>CpG</td>
<td>aerosol</td>
<td>20.4 ± 2.1</td>
<td>0/4</td>
<td>(0)</td>
<td>43.5 ± 16.5</td>
</tr>
<tr>
<td>rSEB + CpG</td>
<td>injection (im)</td>
<td>16.0 ± 0.5</td>
<td>4/4</td>
<td>(100)</td>
<td>-</td>
</tr>
<tr>
<td>rSEB + CpG</td>
<td>aerosol</td>
<td>15.8 ± 2.1</td>
<td>2/4</td>
<td>(50)</td>
<td>43.5 ± 9.1</td>
</tr>
<tr>
<td>rSEB + CpG</td>
<td>im (p) aerosol (b)</td>
<td>19.6 ± 0.3</td>
<td>2/5</td>
<td>(40)</td>
<td>44.0 ± 5.2</td>
</tr>
<tr>
<td>rSEB</td>
<td>im (p) aerosol (b)</td>
<td>20.7 ± 1.2</td>
<td>4/5</td>
<td>(80)</td>
<td>66.5</td>
</tr>
</tbody>
</table>
Resulting Pathology
(in coll. with MAJ N. Twenhafel, Div. of Pathology)
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Conclusions (primate study)

- aerosol vaccination, compared to the injected vaccine, alone *did not* provide adequate protection against aerosol challenge relative to injected vaccine.
- vaccination by a combination of routes (im prime; aerosol boost) provided:
  - Equivalent anti-SEB IgG and increased S-IgA production when compared to injected cohorts.
  - Comparable protection protection against lethal effects of aerosolized SEB.
  - Provided superior protection in abrogating acute-phase clinical intoxication.
- Vaccine dose & optimization of schedule may be improved in future investigations.
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  - Aerogen Corp.
    - Paul Uster
    - Jim Fink

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**ANIMAL USE STATEMENT**

This research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

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