



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

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Report Documentation Page

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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 \uparrow 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,



Proof of concept: Mouse study

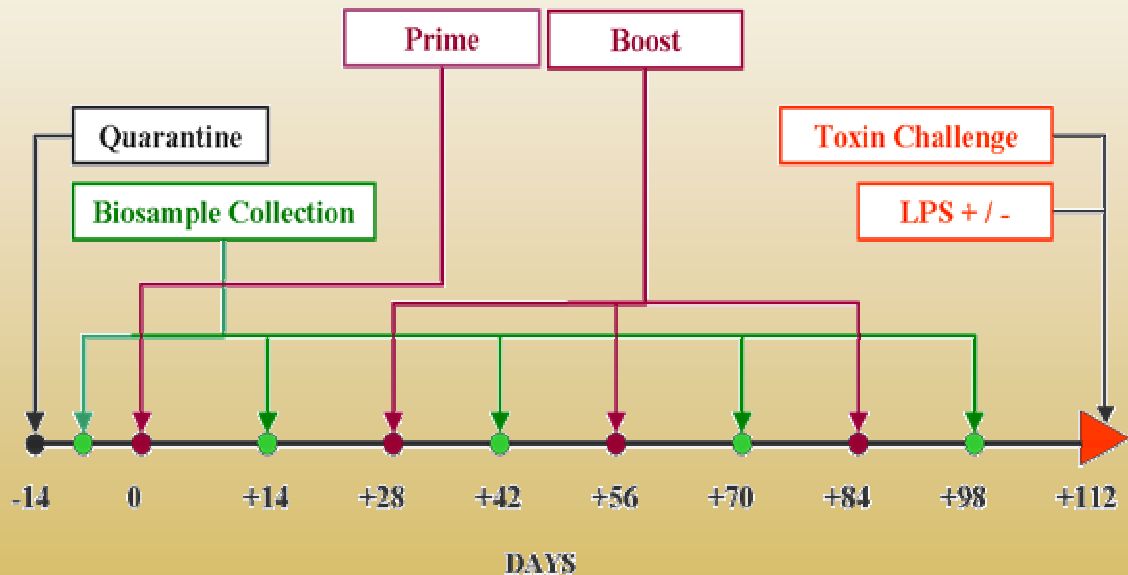
two strains of mice used in study

- 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)



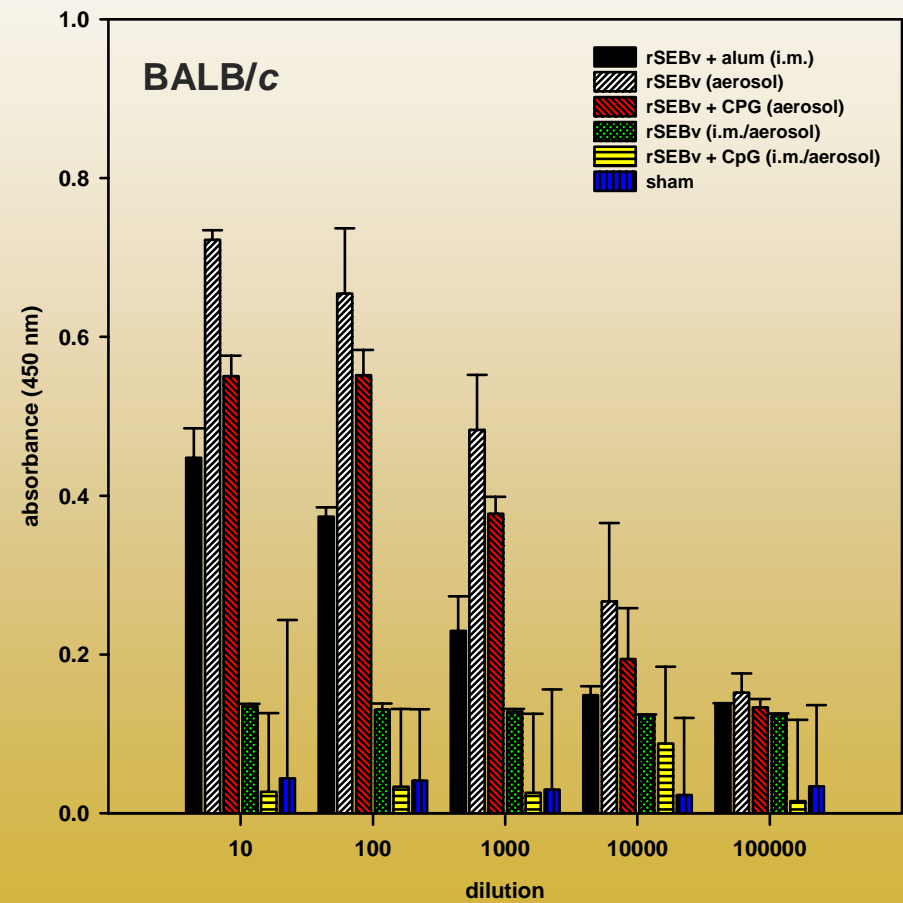
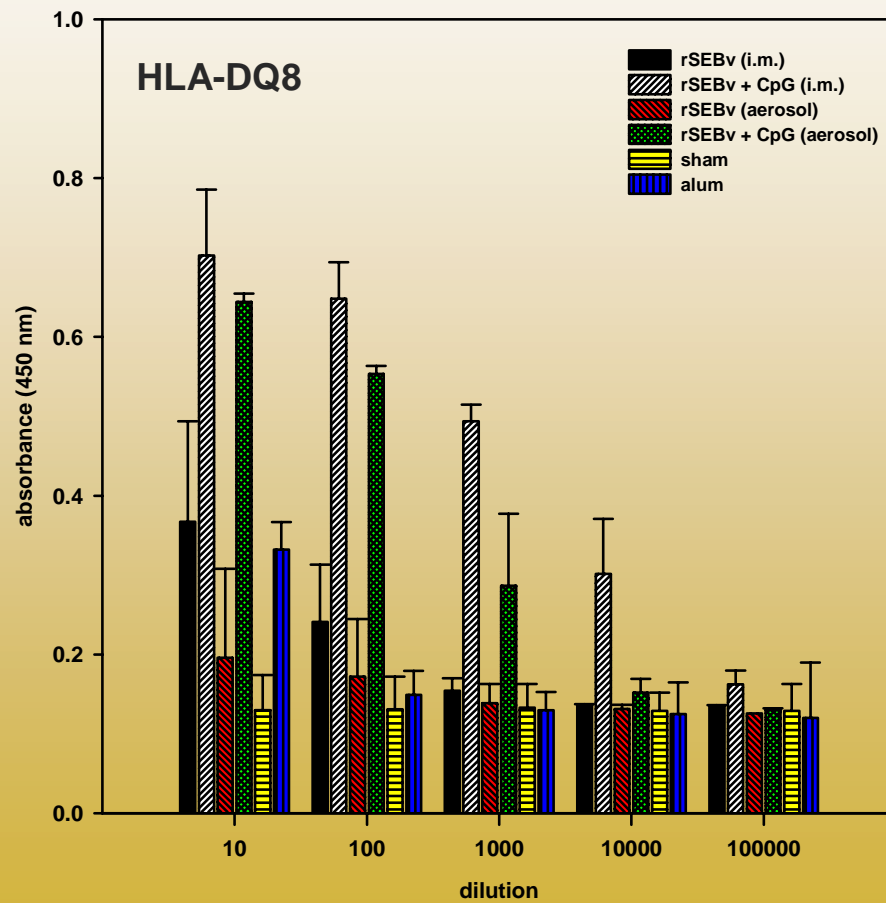
Vaccine dosing and challenge

- 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 LD₅₀s)
 - aerosol
- LPS potentiation
 - 75 μg (IP; BALB/c)



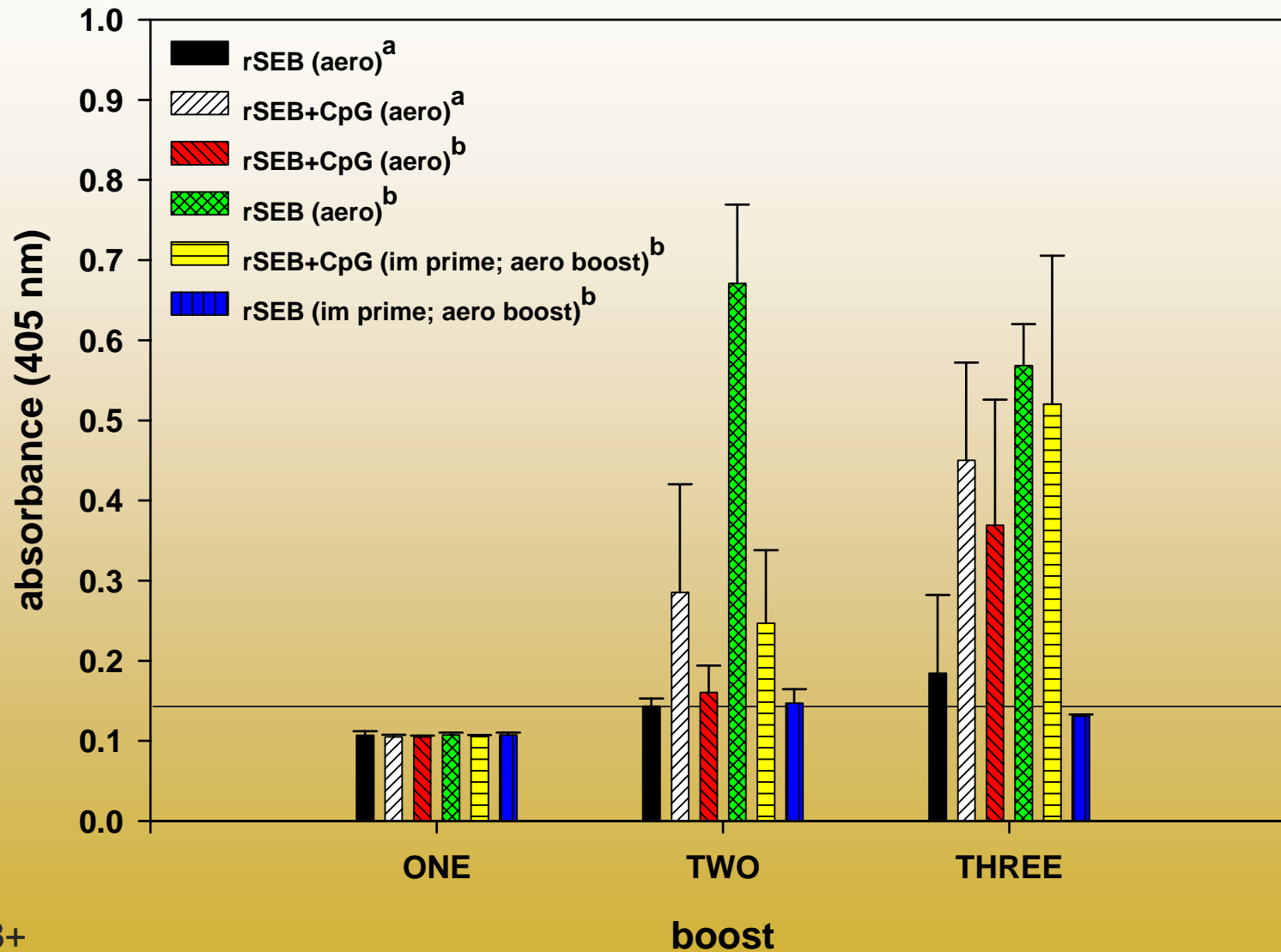


Mouse data: serum IgG





IgA in Saliva



^a DQ8+

^b BALB/c



Mouse Data: Aerosol Challenge

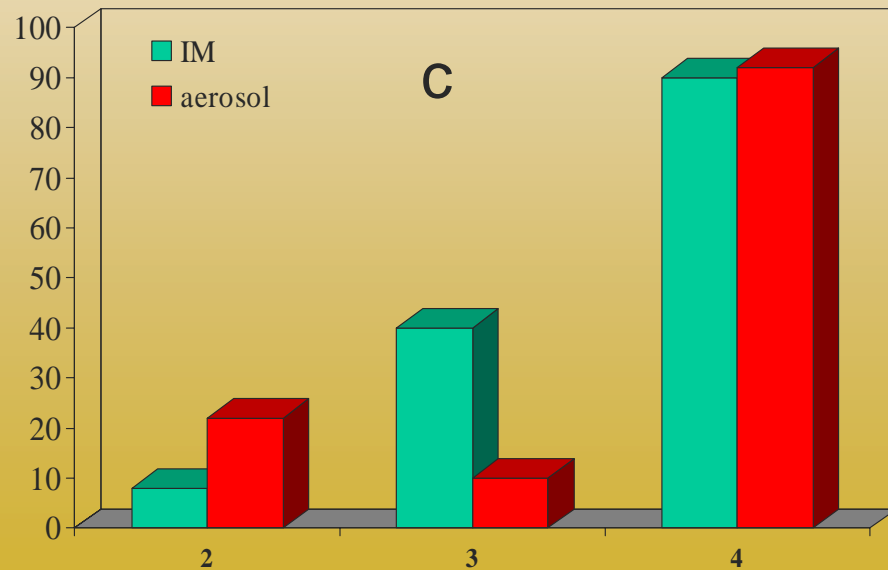
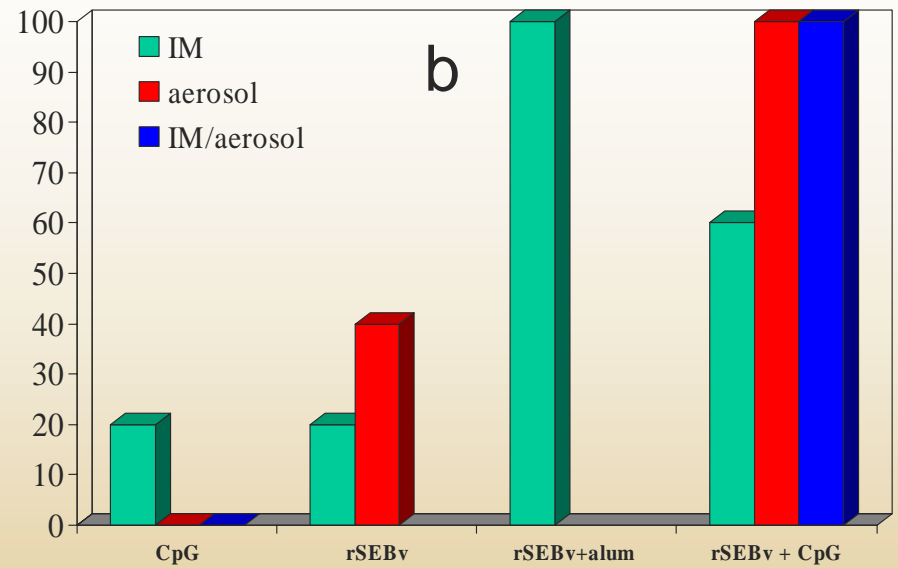
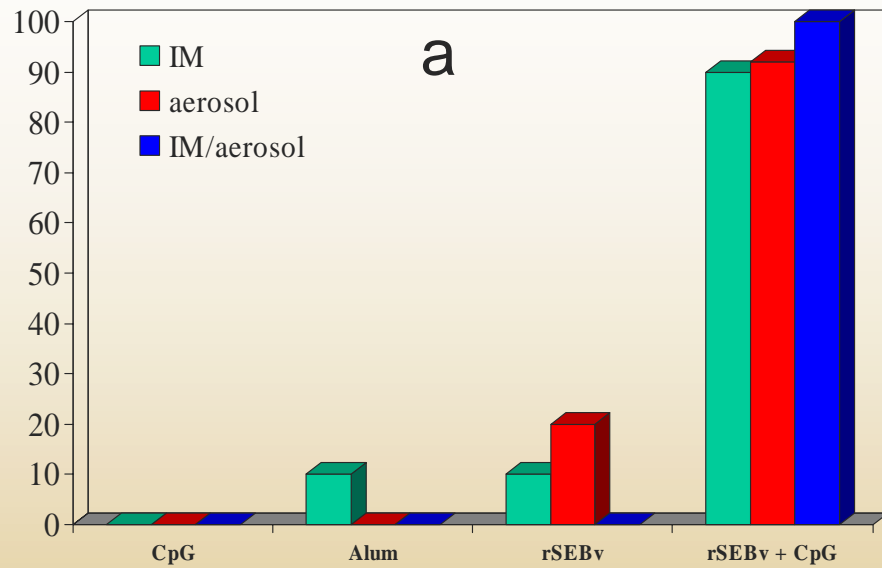


Figure 1. Comparative protection of mice against a lethal dose of aerosolized SEB in BALB/c (a) or HLA-DQ8 transgenic (b). Optimization of vaccine schedule (c) indicated four vaccinations provided optimal protection in the BALB/c mouse.



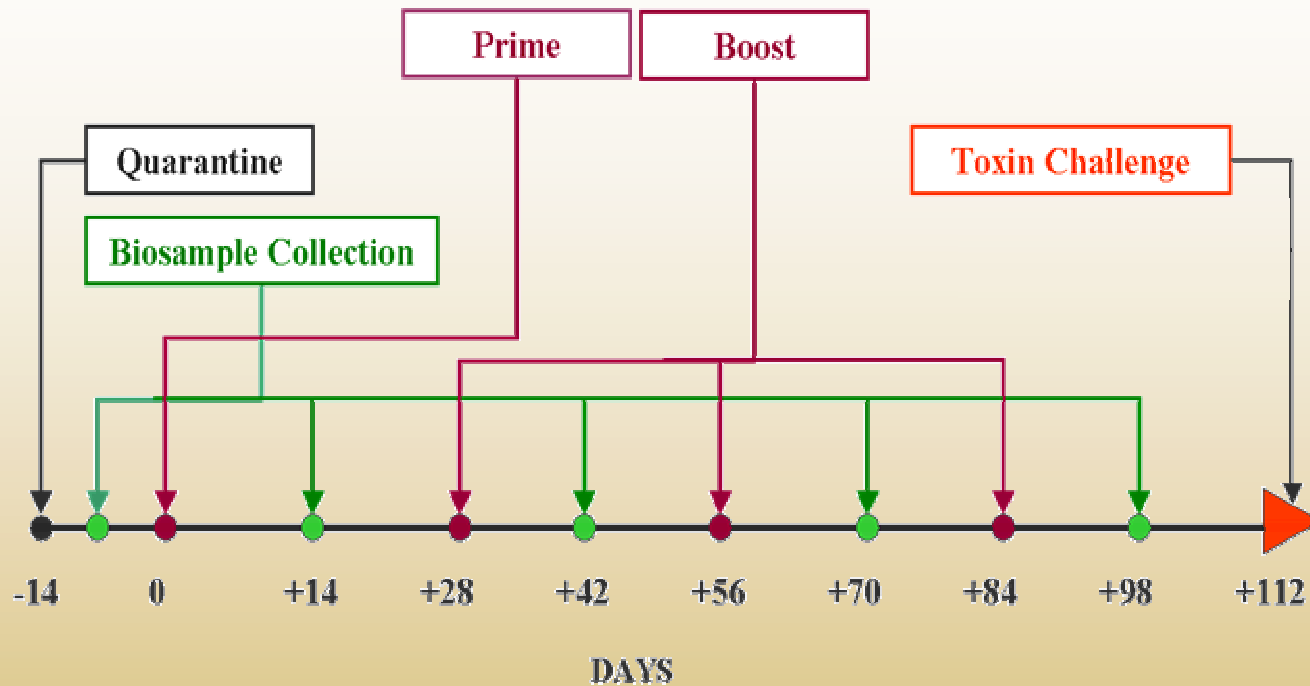
Primate study

- 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 μ m)

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.

Group	Vaccination	Administration route
1	Sham	IM
2	CpG ^b	aerosol
3	rSEBv ^a + CpG ^b	IM
4	rSEBv ^b + CpG ^b	aerosol

Phase II

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

Group	Vaccination	Administration route
1	rSEBv ^a	IM prime; aerosol boost
2	rSEBv ^a + CpG ^b	IM prime; aerosol boost

^a 20 µg/vaccination (measured)

^b 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



Nebulizers		• Clinic use
Inhalers		• Field Use



*provided via USAMRIID CRADA with Aerogen Corp.

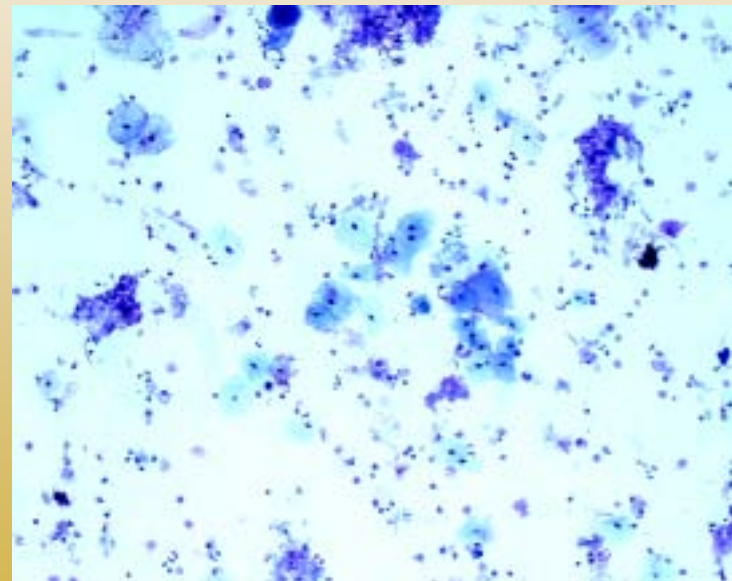
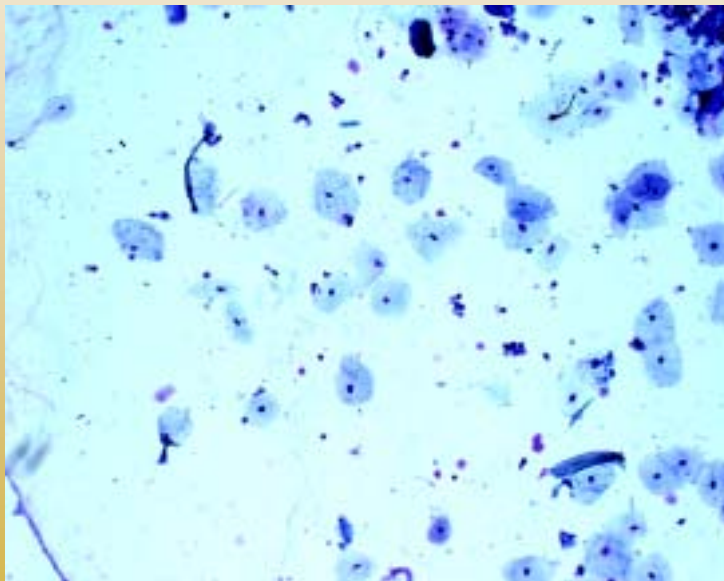


Acute Pulmonary toxicity



Lung lavage

– CpG Oligo 10103 delivered by aerosol. Photos at 100x. (a) preexposure, (b) 24 hours postexposure.





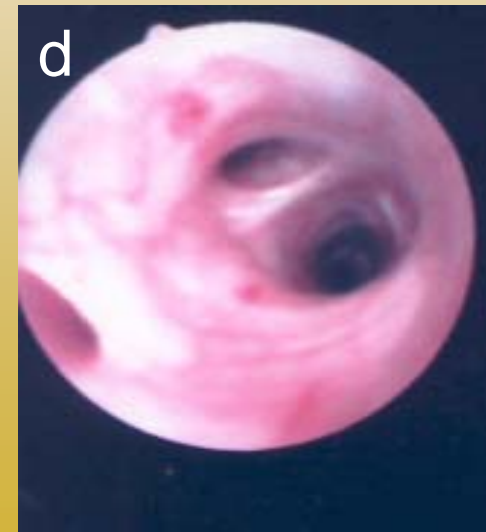
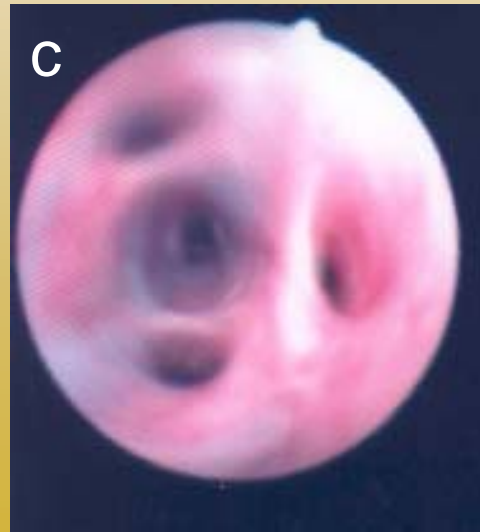
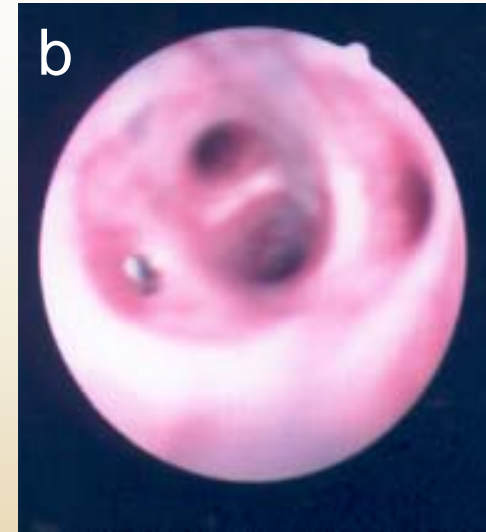
Bronchoscopies/BAL



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).

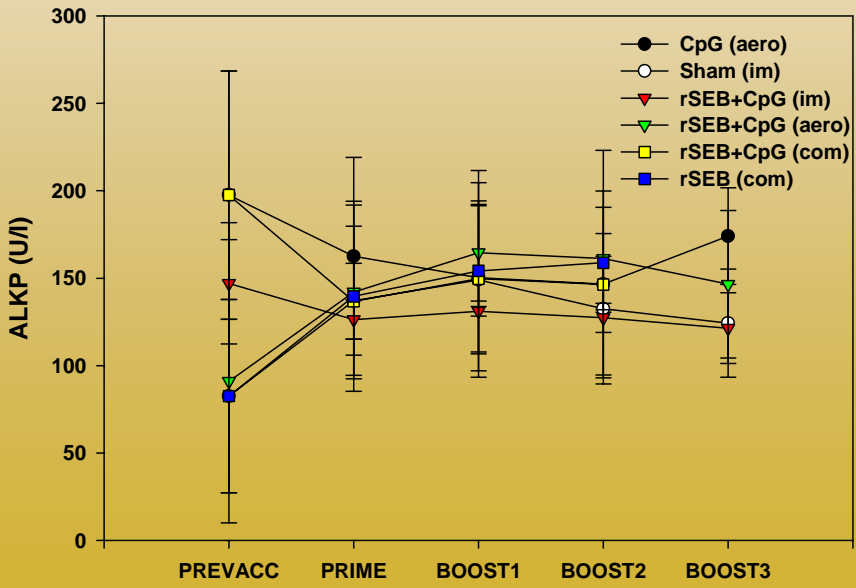
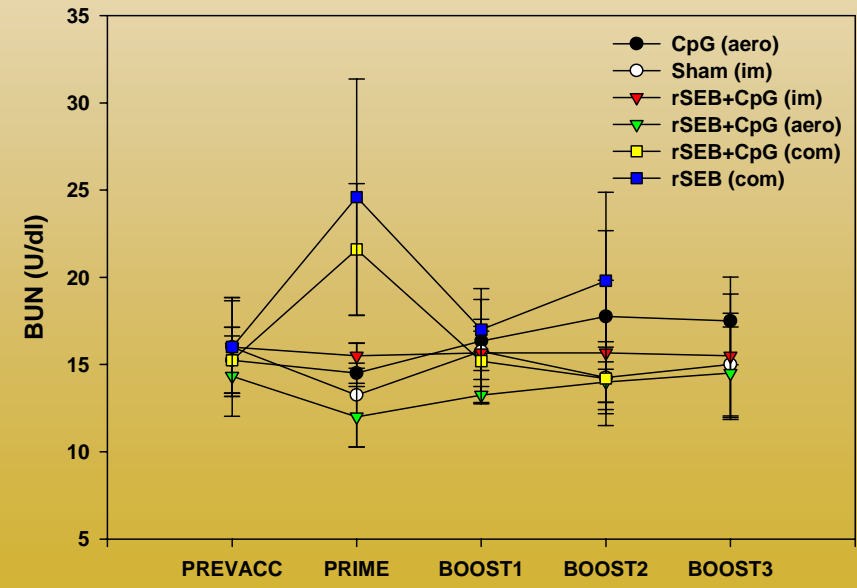
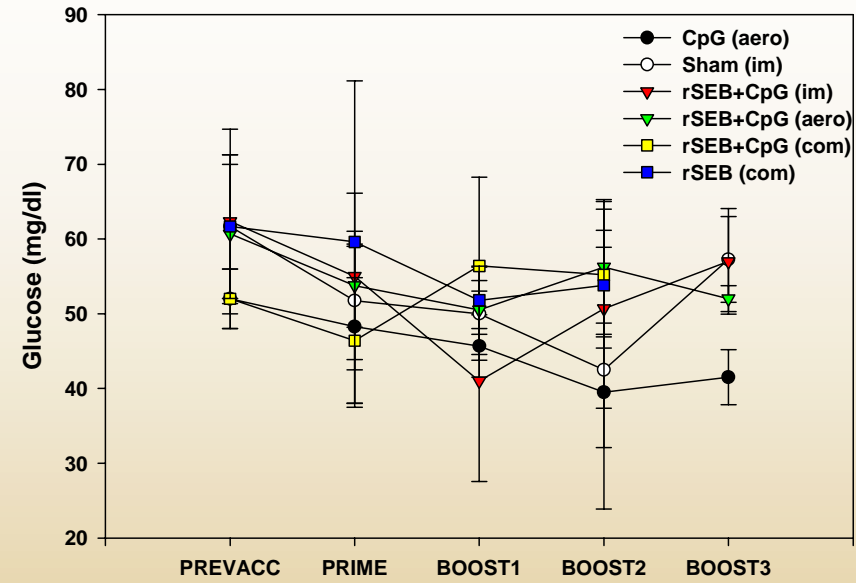
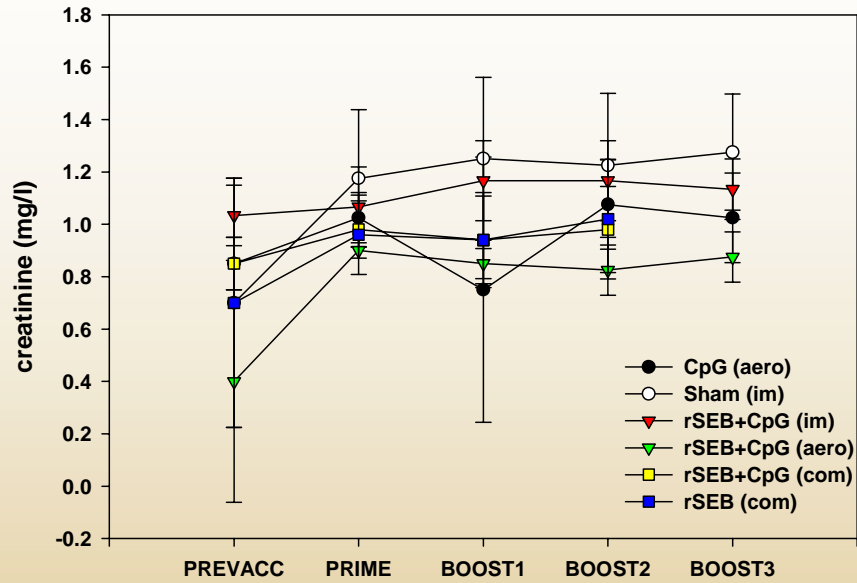
Results of visual observations:

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



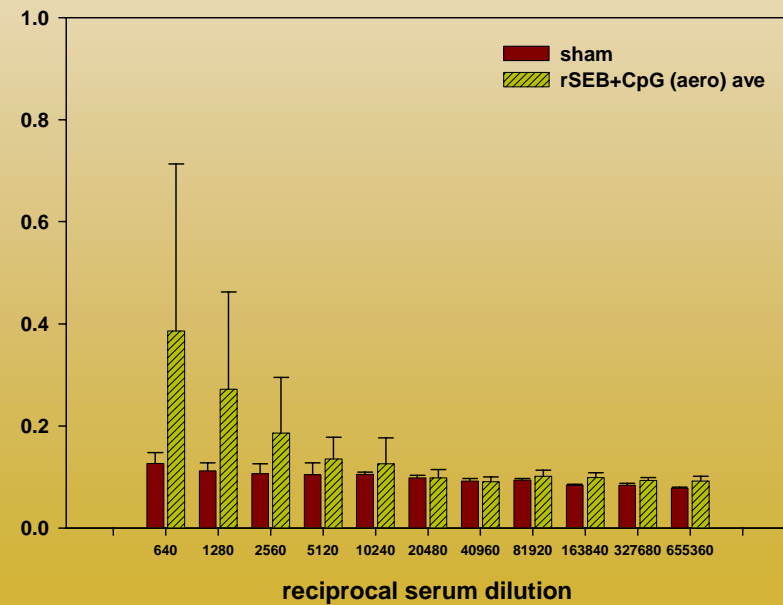
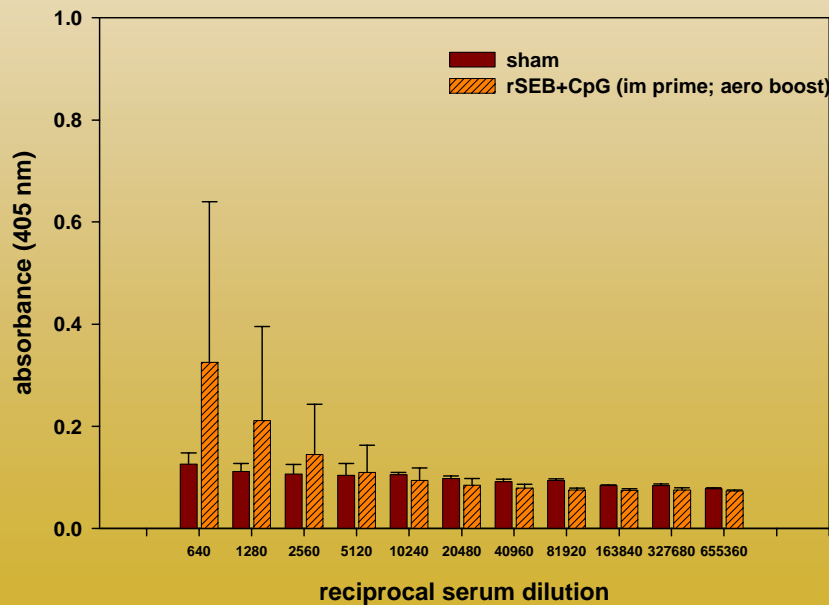
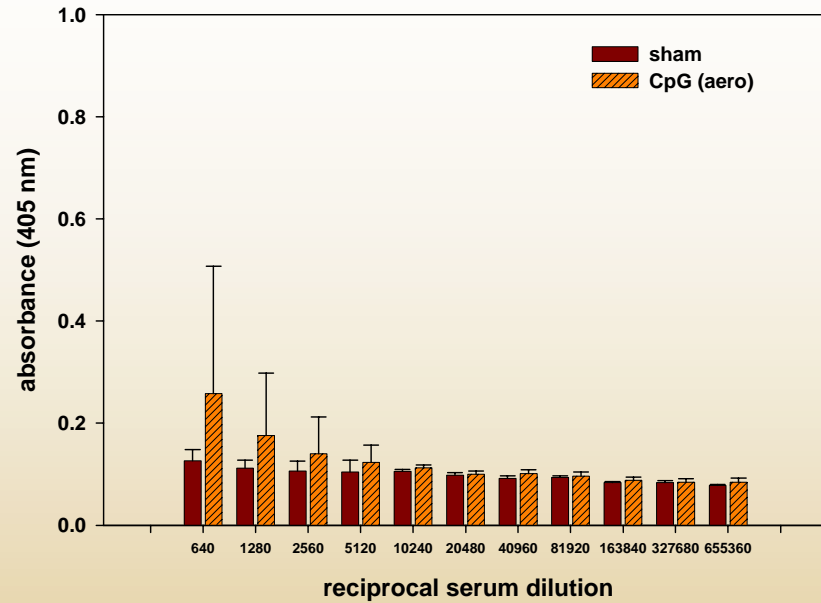


clinical chemistries



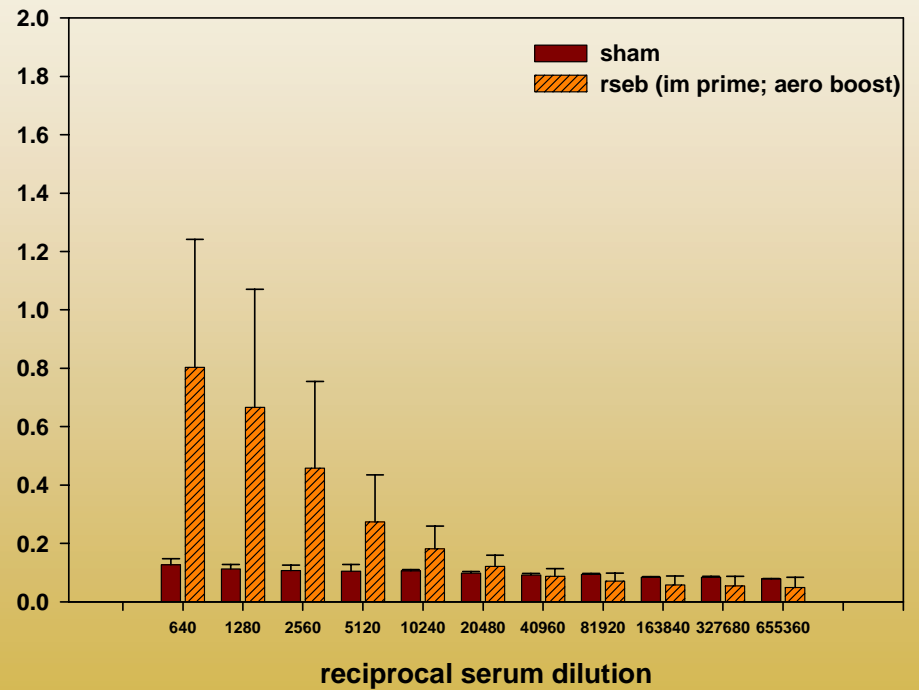
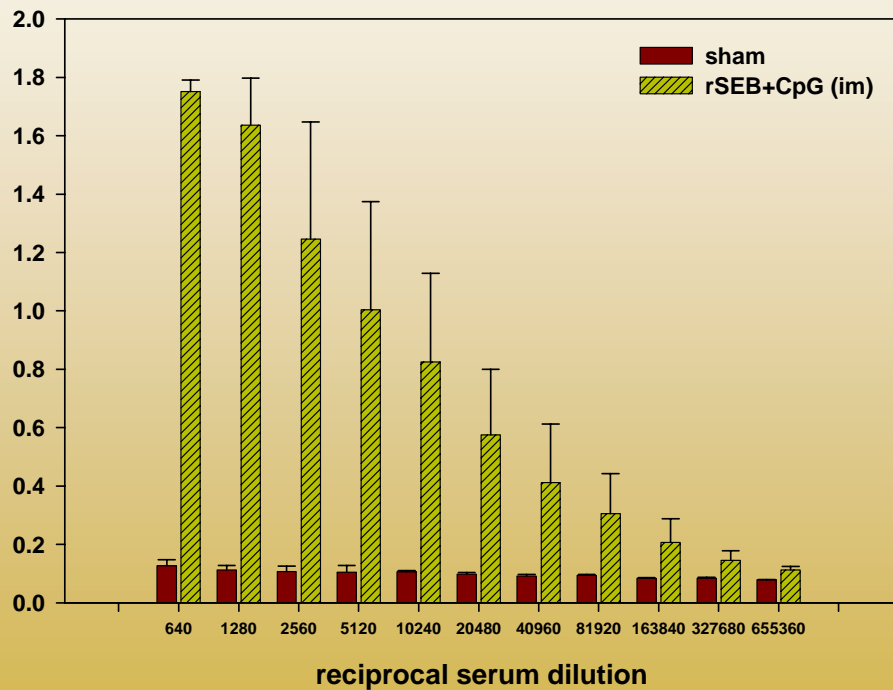


Serum Anti-SEB IgG development in rhesus monkeys one week before aerosol challenge using either rSEB alone, CpG ODN alone, or comixed administered either by injection, inhalation, or combination of routes thereof.



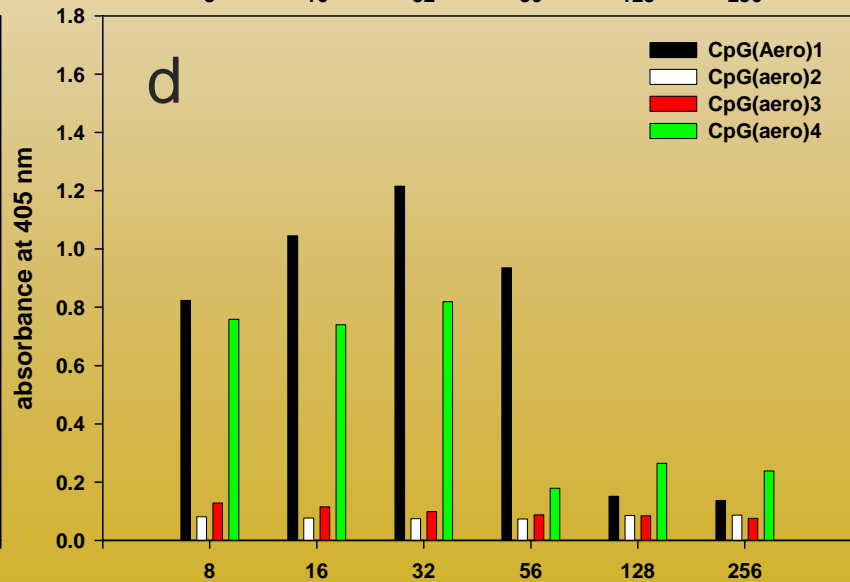
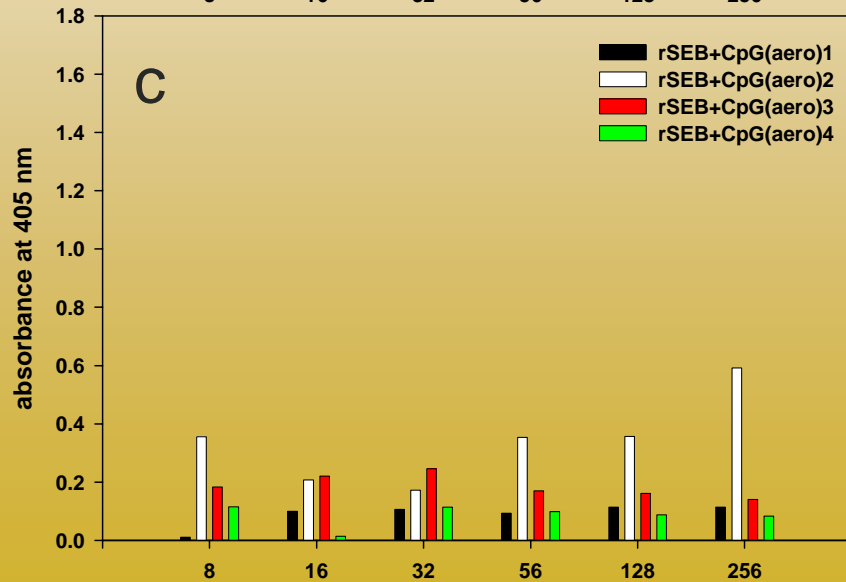
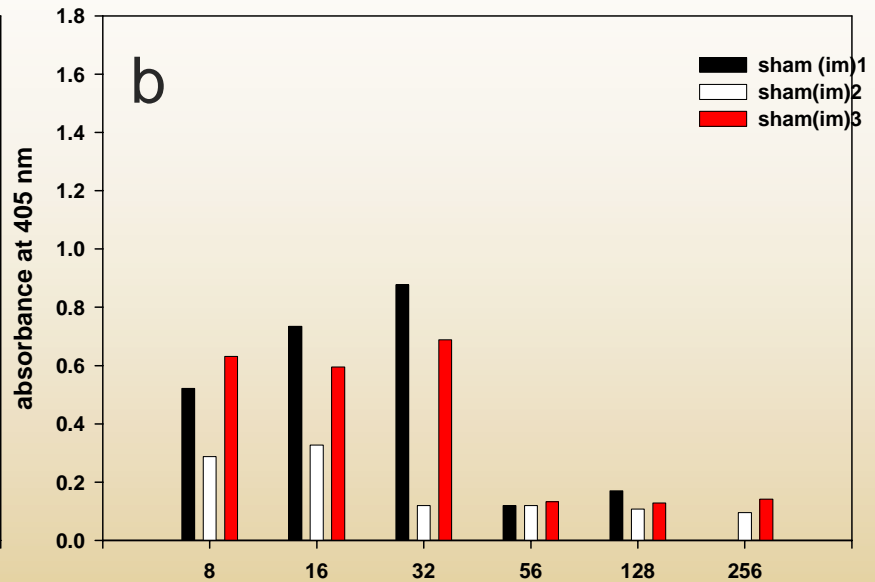
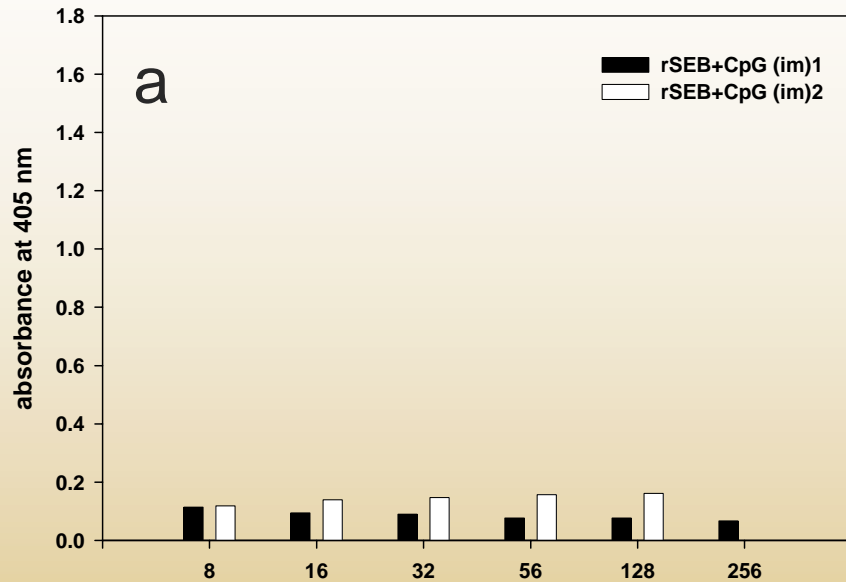


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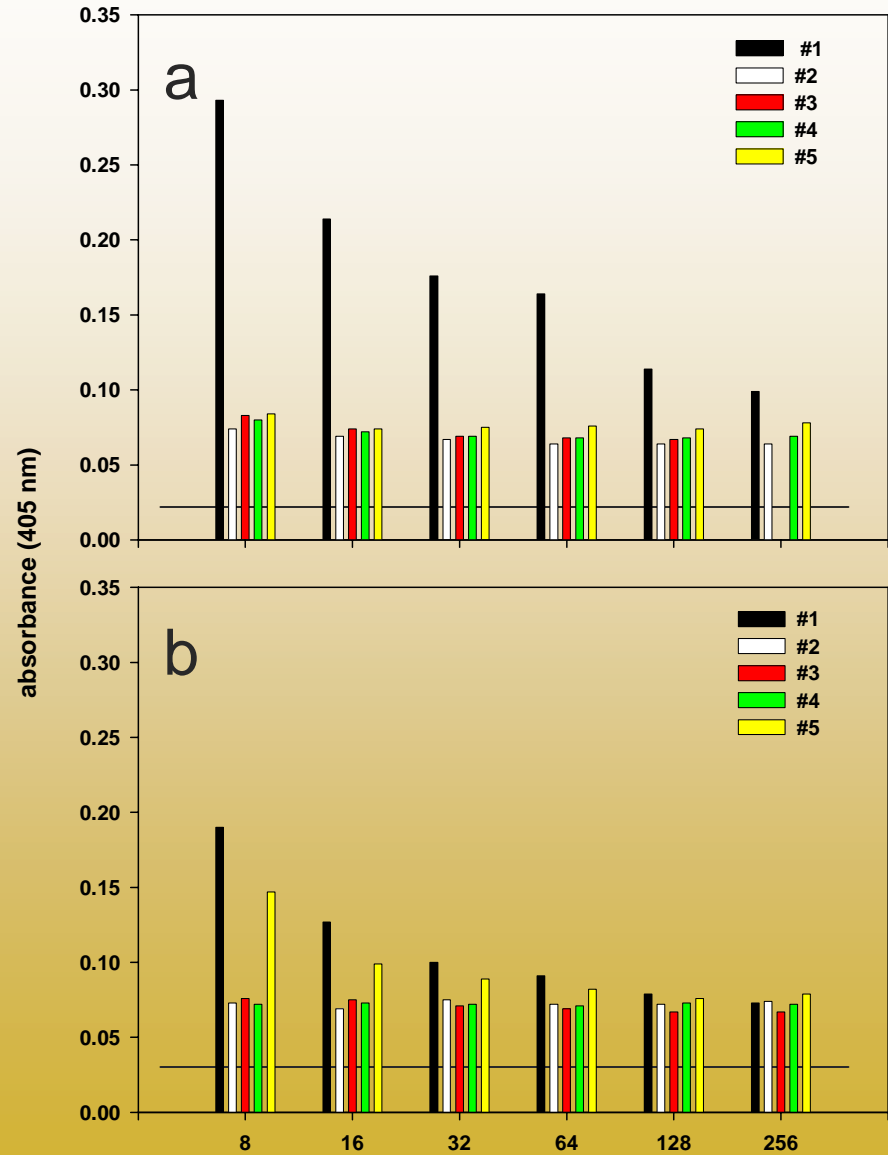


S-IgA development in rhesus monkeys one week before aerosol challenge (+98d).
Individual values are presented for the following groups: (a) rSEB+CpG (im), (b) sham (im); (c) rSEB + CpG ODN (aerosol), and (d) CpG ODN (aerosol).





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

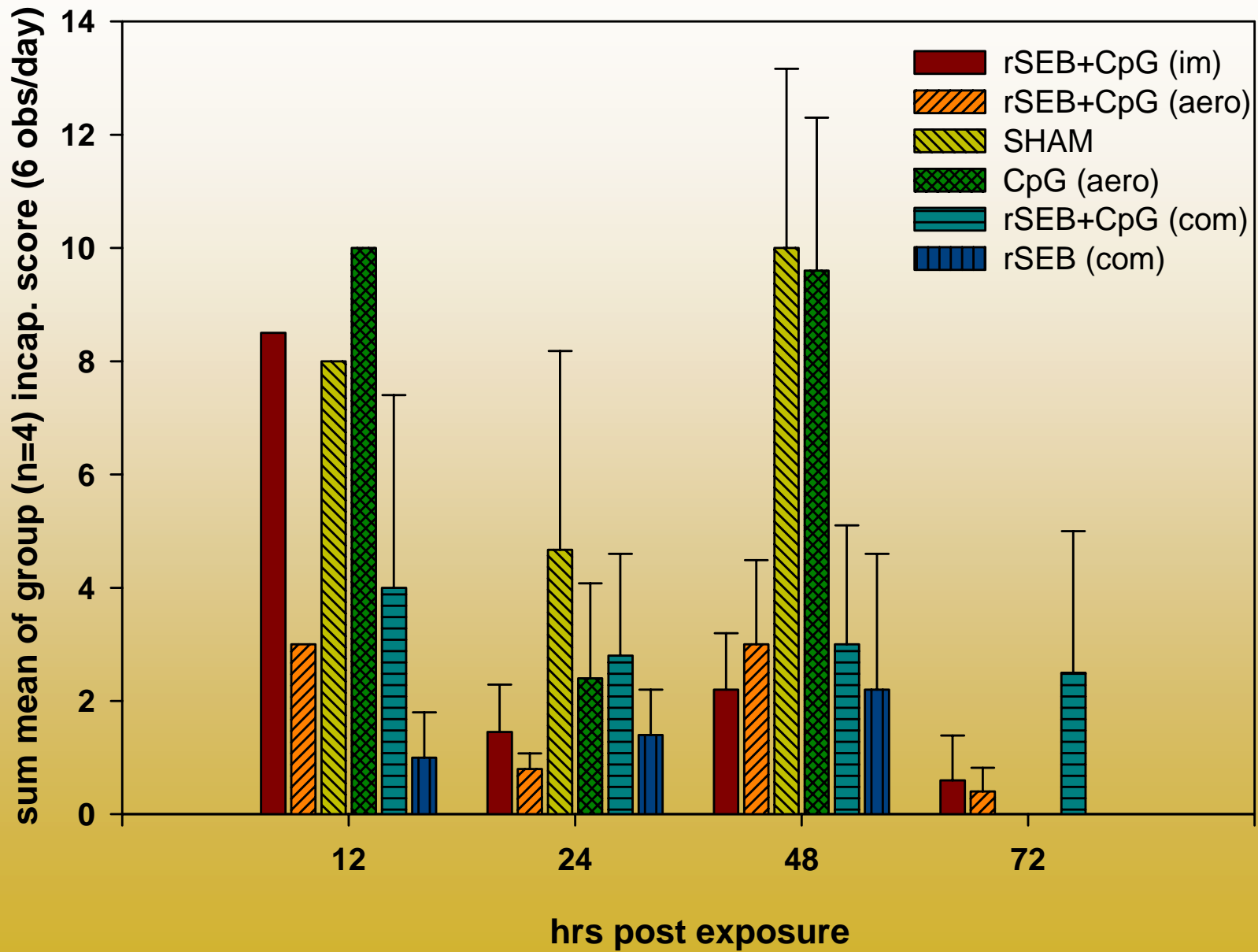
- 0 NO EVIDENCE OF SYMPTOMS
- 1 PRESENCE OF SYMPTOM IN A MILD OR UNDETERMINED DEGREE
- 2 INDICATES A DEGREE NOTABLE FOR REQUIRING THERAPY
- 3 SEVERE

- Signs were graded for each animal

<u>SIGN</u>	<u>DESCRIPTION</u>
VOMITING/HEAVING	ACTIVE VOMITING OR WRETCHING; DRY HEAVING
COUGH	ANY SUDDEN EXPELLING OF AIR FROM THE LUNGS INDICATIVE OF A COUGH
MALAISE	LACK OF MOVEMENT RELATIVE TO PRE-EXPOSURE LEVEL
WEAKNESS	DIFFICULTY GRABBING ON TO CAGE; OR ABILITY TO HOLD UP ARMS
ANOREXIA	COUNT BISCUITS CONSUMED BASED ON CARETAKER FEEDING SCHEDULE
MUCOUS MEMBRANES	DRYNESS CHANGES
LABORED BREATHING	ACTIVE EXPIRATION; DIFFICULTY WITH INHALATION

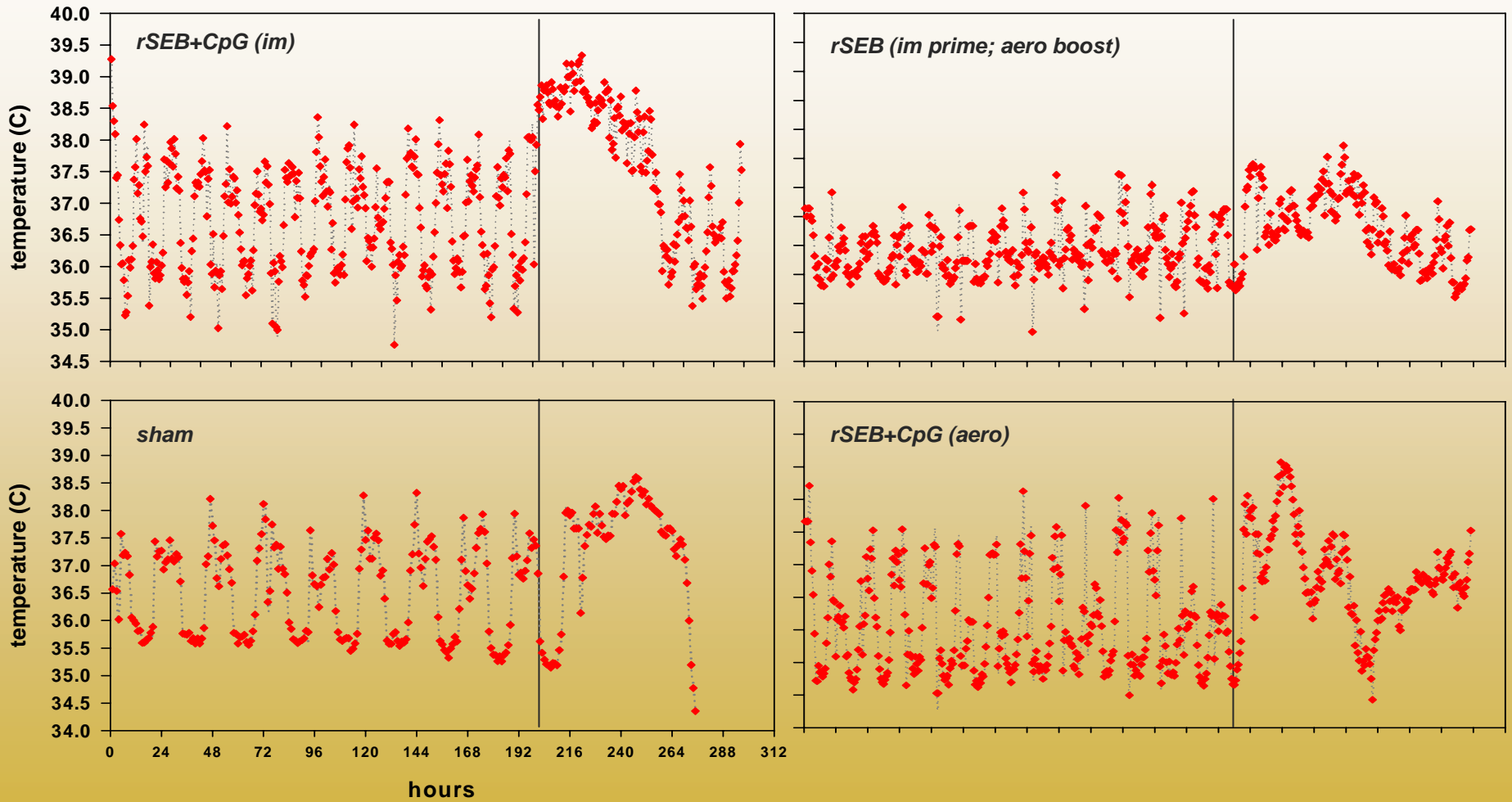


Clinical Outcome Results (Phases I & II)





Telemetry: Physiological Response to SEB challenge





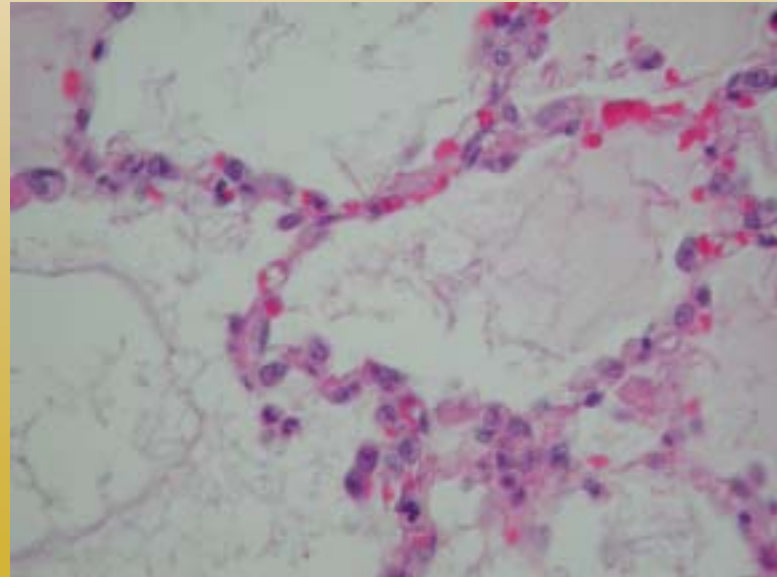
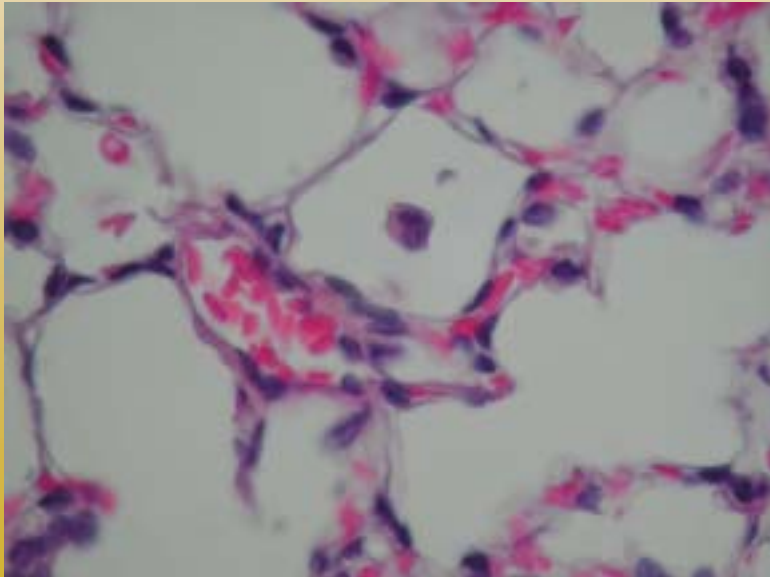
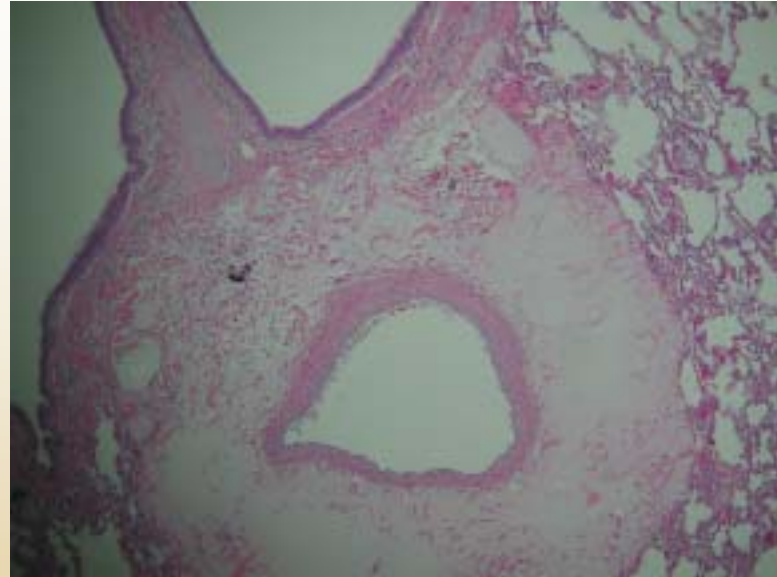
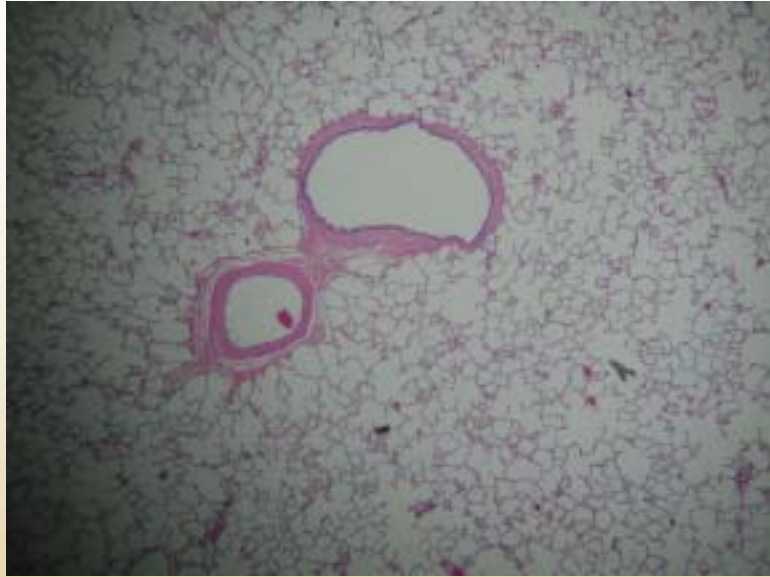
primate survival



groups		challenge	survival		
Treatment	Vacc. Route	LD50s (mean \pm s.d.)	alive/grp	(%)	MTD (hr)
Sham	injection (im)	16.0 \pm 2.5	0/4	(0)	43.0 \pm 12.9
CpG	aerosol	20.4 \pm 2.1	0/4	(0)	43.5 \pm 16.5
rSEB + CpG	injection (im)	16.0 \pm 0.5	4/4	(100)	-
rSEB + CpG	aerosol	15.8 \pm 2.1	2/4	(50)	43.5 \pm 9.1
rSEB + CpG	im (p) aerosol (b)	19.6 \pm 0.3	2/5	(40)	44.0 \pm 5.2
rSEB	im (p) aerosol (b)	20.7 \pm 1.2	4/5	(80)	66.5

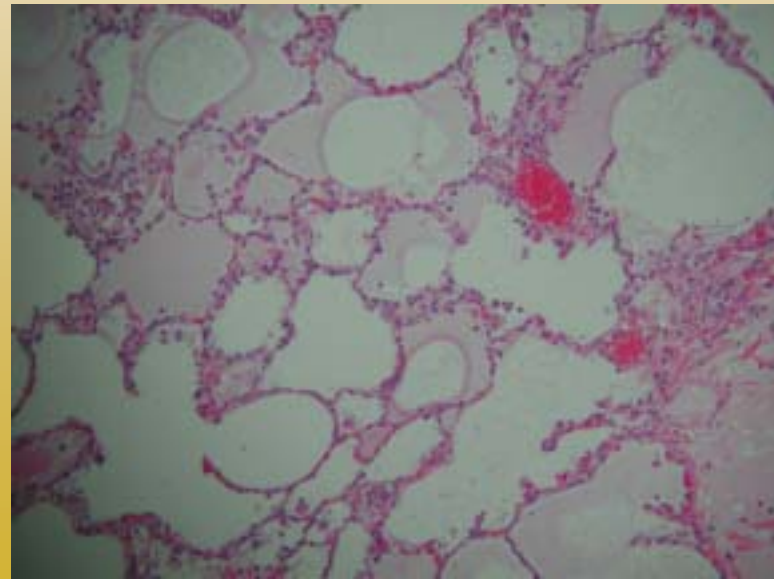
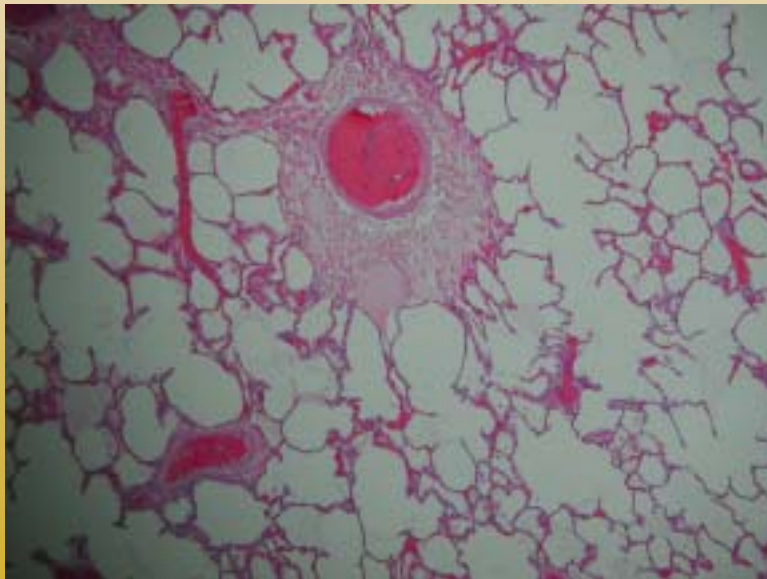


Resulting Pathology
(in coll. with MAJ N. Twenhafel, Div. of Pathology)





Resulting Pathology
(in coll. with MAJ N. Twenhafel, Div. of Pathology)





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 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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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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