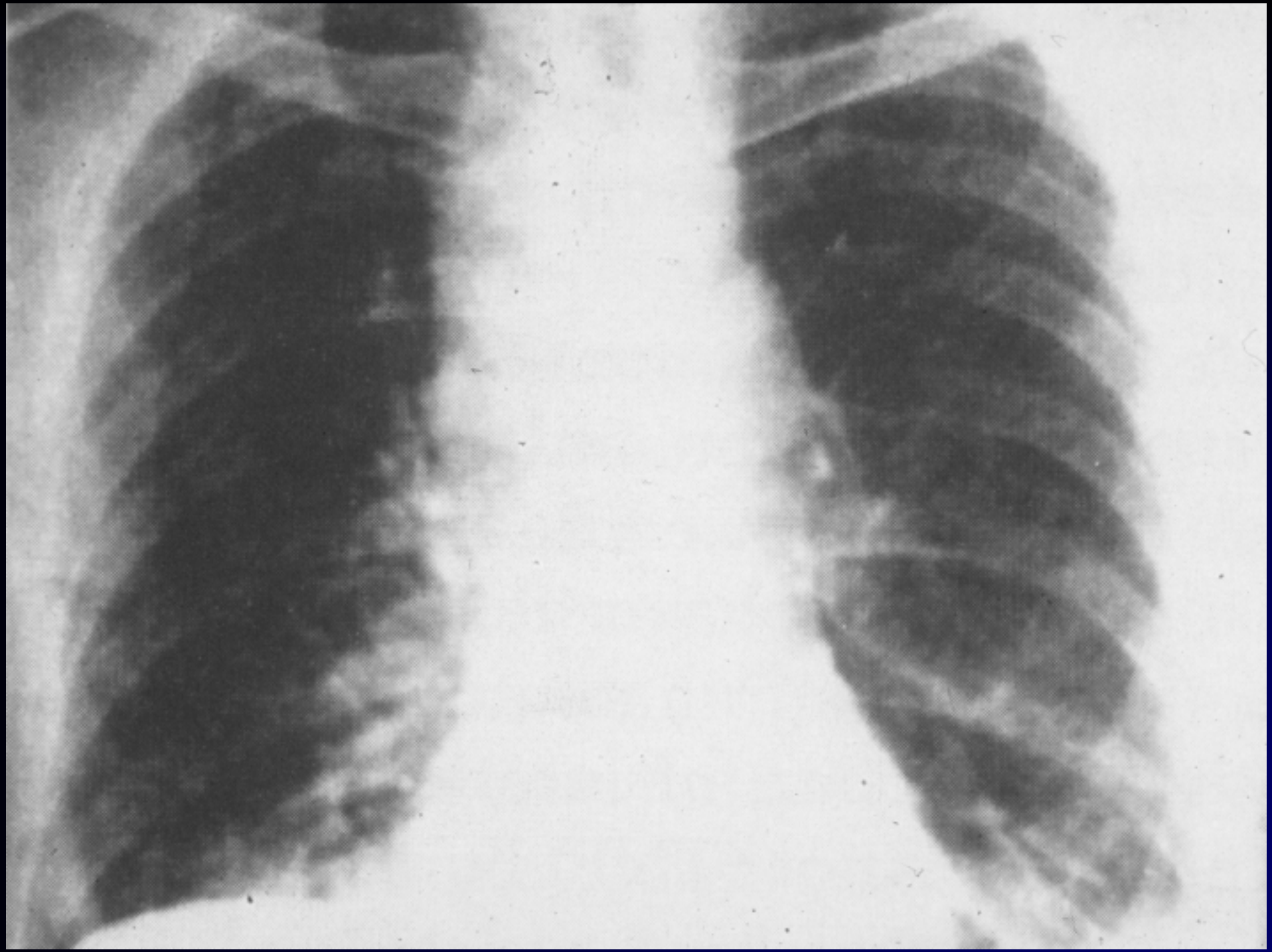


History of early studies on anthrax

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

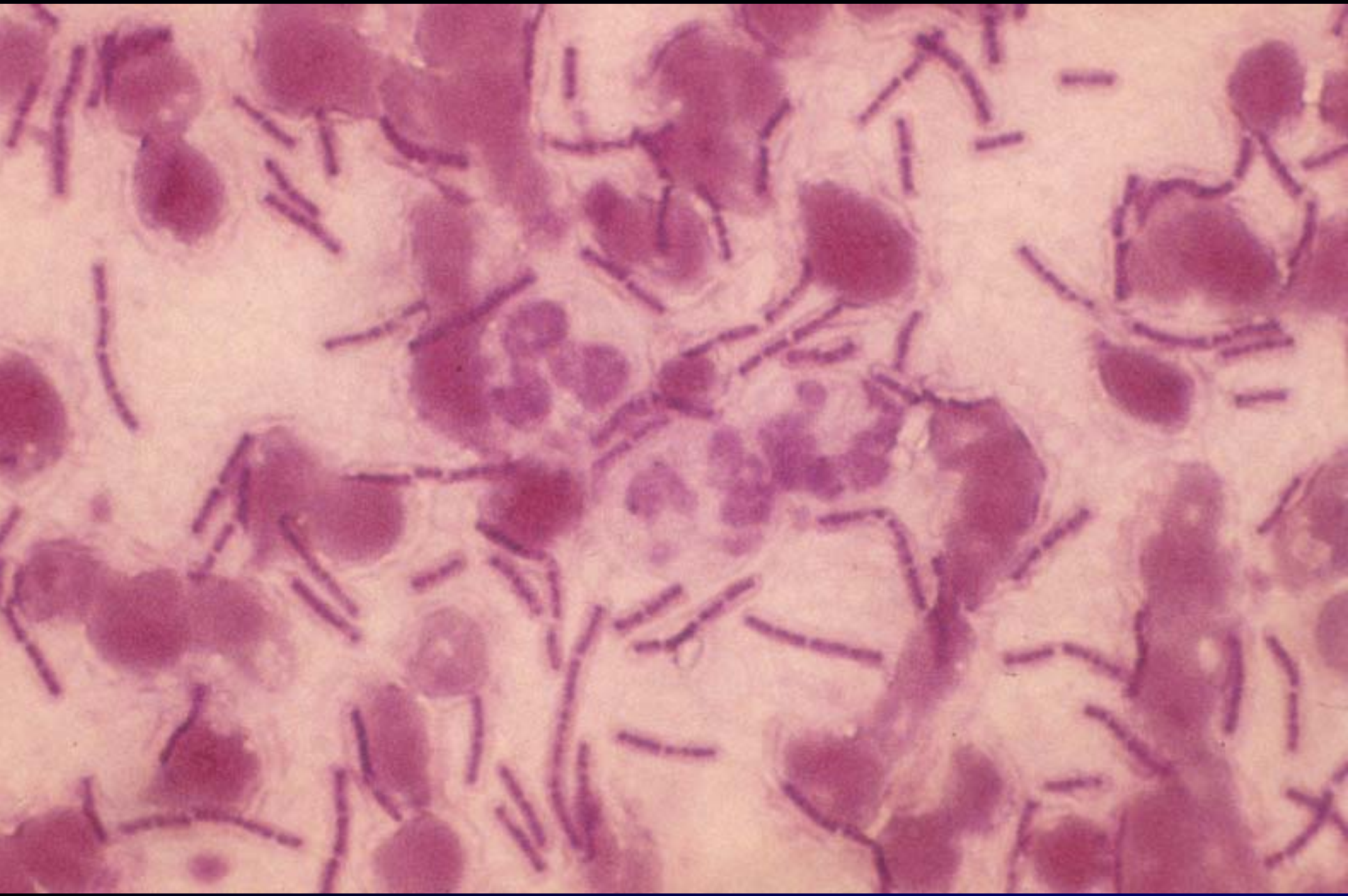
Disease of Antiquity

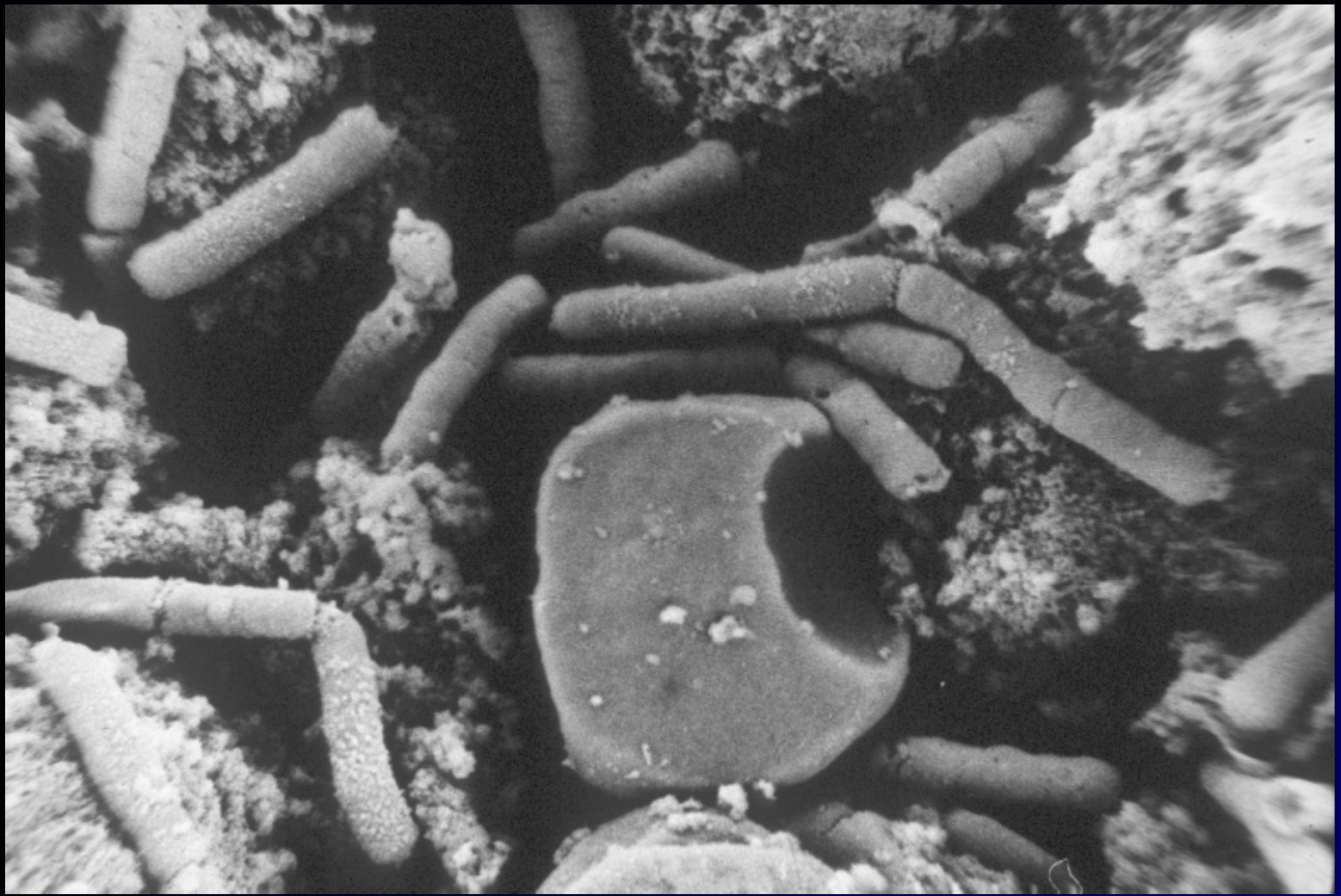
1877 - The first disease for which a microbial etiology was determined (Koch)

1881 - Live vaccine developed (Pasteur)

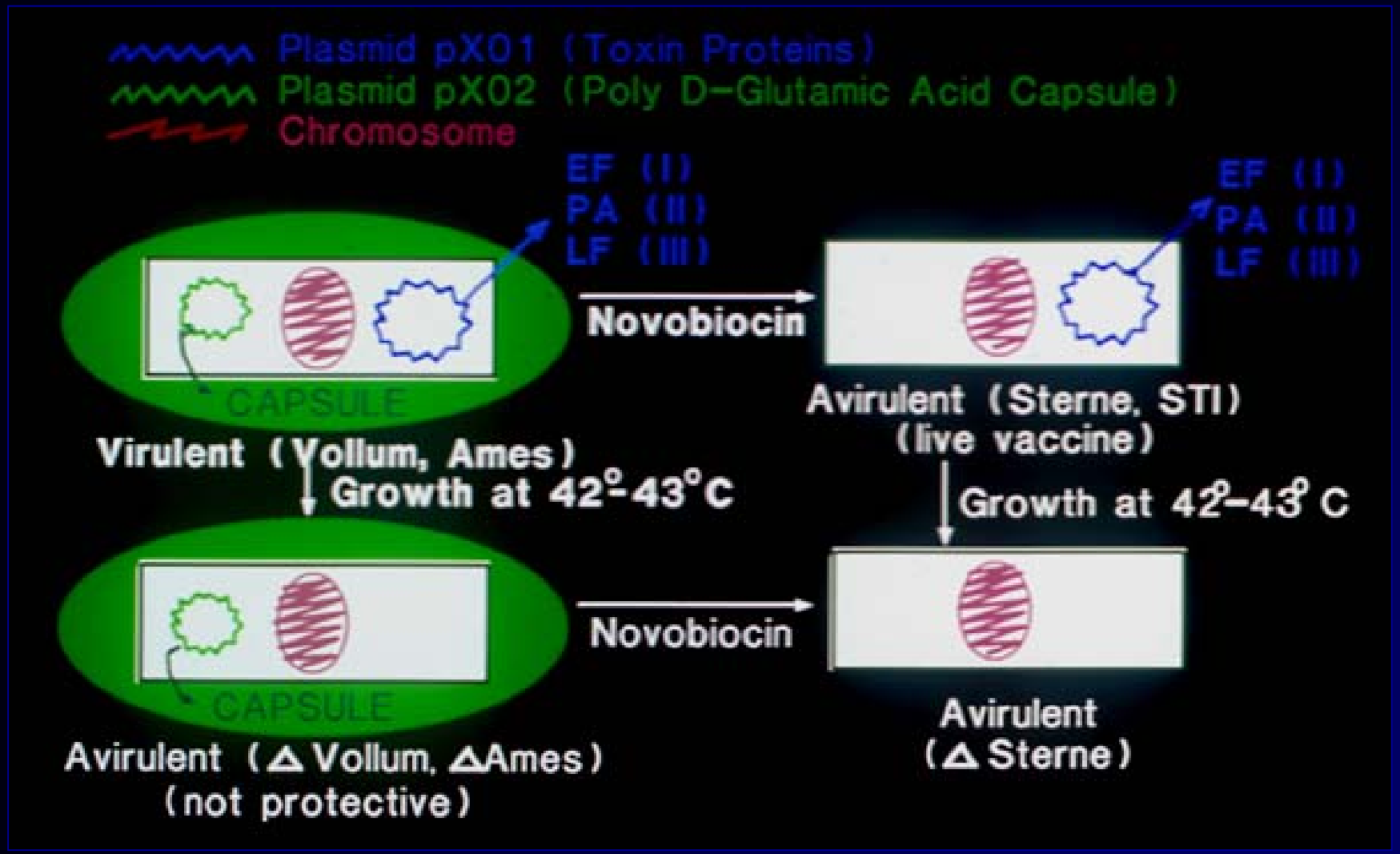
Organism

- Gram-positive, sporeforming, non-motile bacillus
- Virulence factors:
  - Polyglutamic acid capsule
  - Lethal toxin
  - Edema toxin





Variant Strains of *Bacillus anthracis*



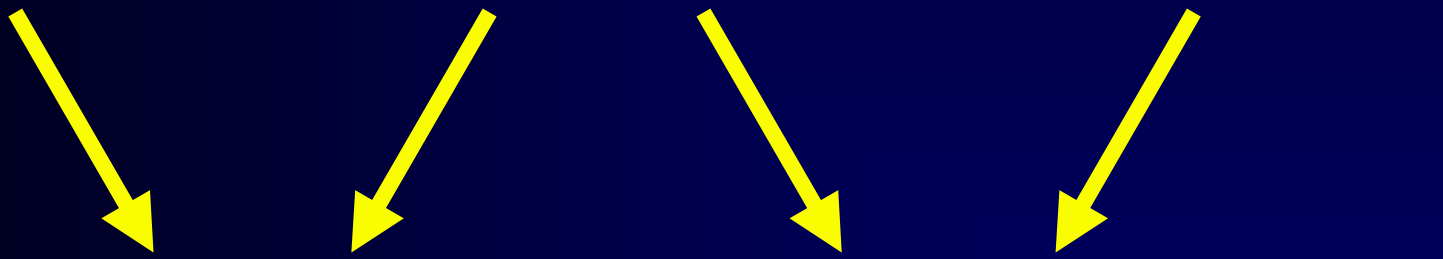
Anthrax Toxin Components

Activities, Properties, Nomenclature

Edema Factor
(EF)
MW 89,000
Adenylate Cyclase

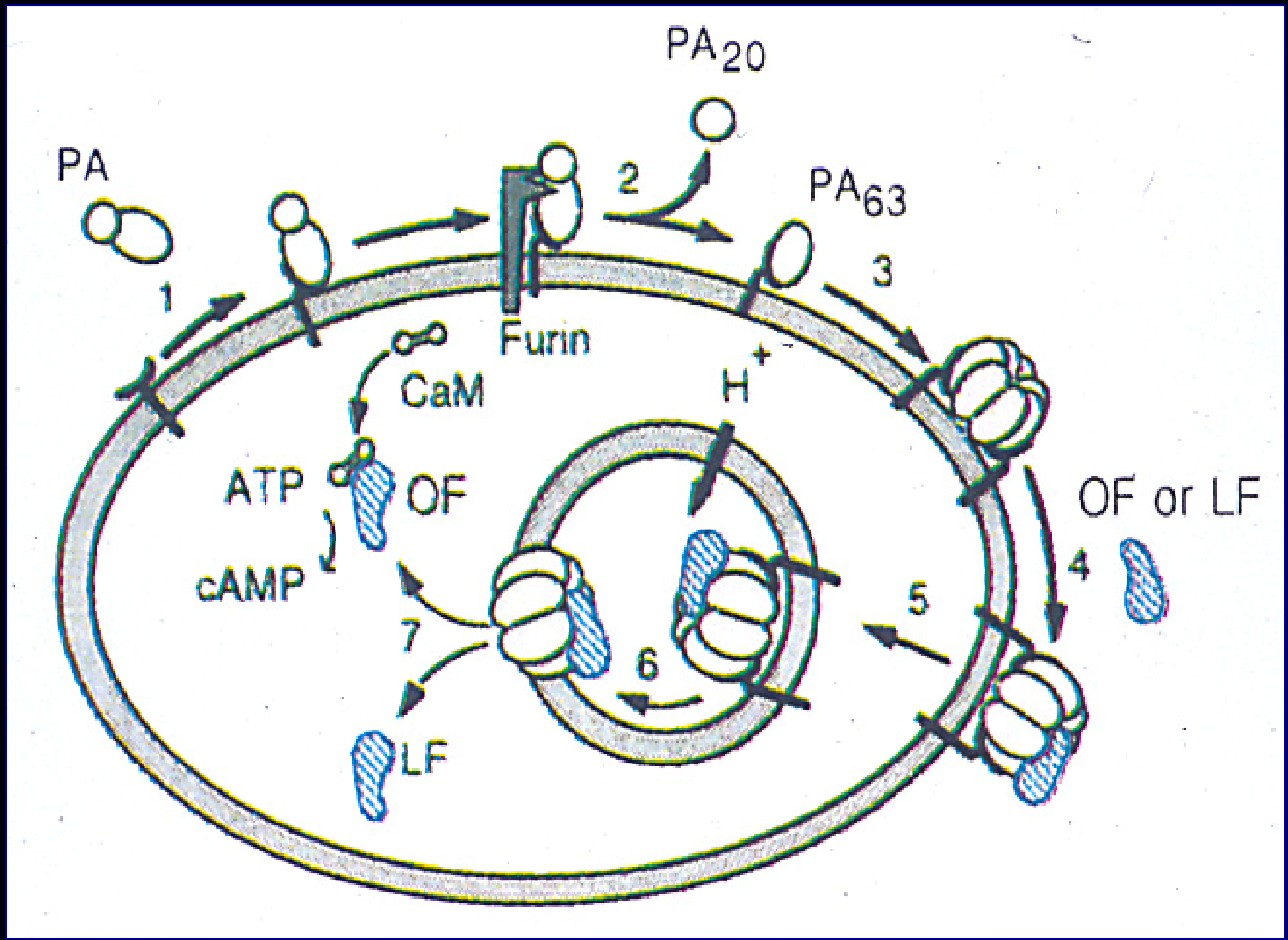
Protective Antigen
(PA)
MW 83,000

Lethal Factor
(LF)
MW 90,000
metalloprotease



Edema in Skin
(Rabbits, Guinea Pigs)
Increased Cyclic AMP

Lethality
(Rats, Mice, etc.)
Macrophage Lysis


PA20
PA
PA63
2
1
3
Furin
CaM
H+
ATP
OF
OF or LF
cAMP
4
5
7
6
LF

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

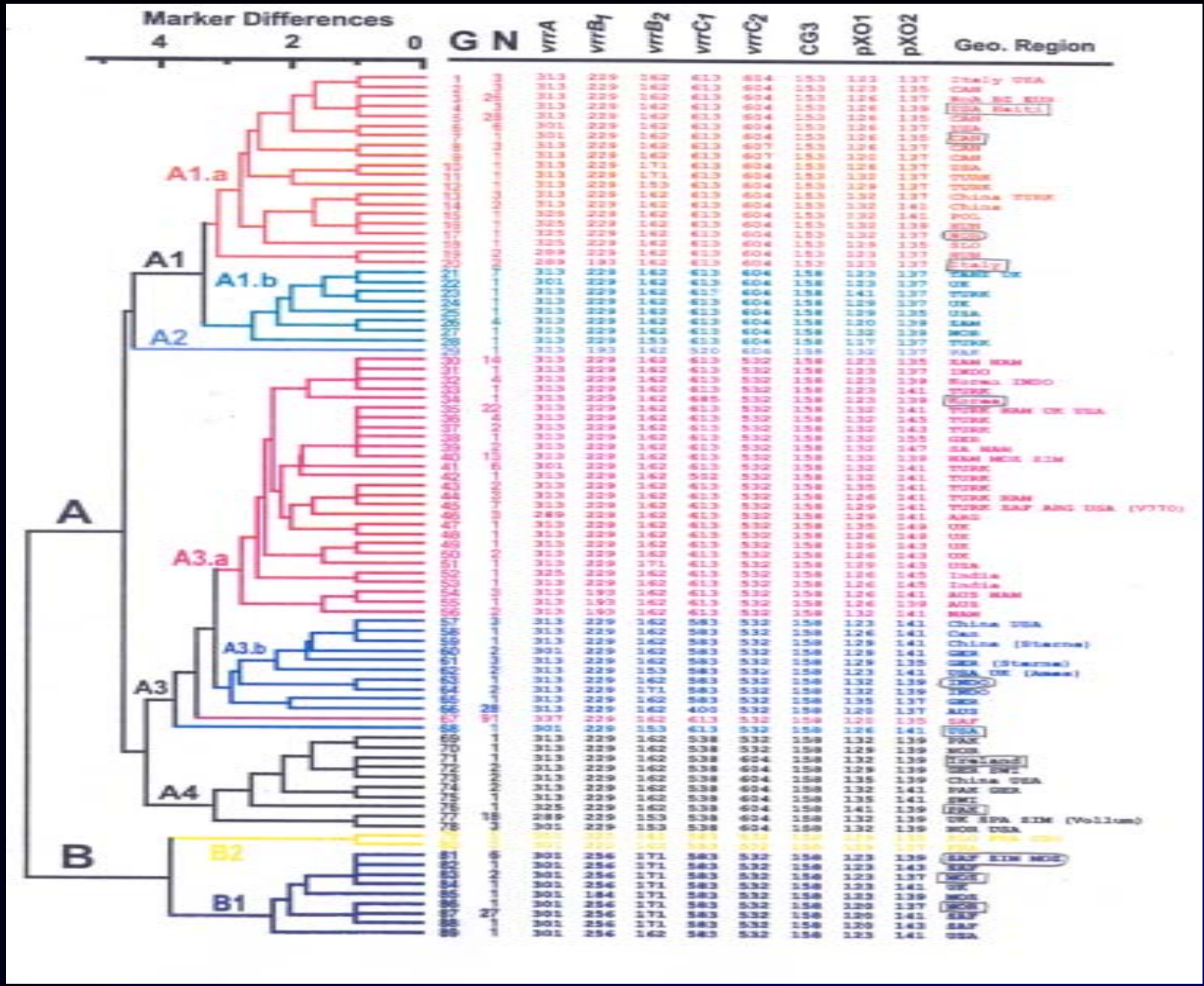
Efficacy of AVA in Guinea Pigs

Strain/Origin	Surv/Total (%)	vrrA[1]	MLVA[2]
Human Isolates			
ASIL K4539 / France	1 / 16 (6)	3	
BA1086 / Zimbabwe	1 / 15(7)	3	
ASIL K5926 / India	2 / 16(13)	5	
ASIL K7038 / S. Korea	3 / 16(19)	5	A3
ASIL K6387 / India	4 / 16(25)	5	
28 Ohio ASB / USA	5 / 16(31)	3	A3
BA1017 / Haiti	5 / 16(31)	4	
BA1023 / Pakistan	8 / 16(50)	5	A4
Other Isolates			
33 / South Africa	2 / 16(13)	3	B1
ASIL K1769 / South Africa	4 / 16(25)	6	
BA1024 / Ireland	7 / 16(44)	4	A4
BA1033 / South Africa	16 / 16(100)	4	

Strain & Origin	Surv/Total (%)	vrrA[1]	MLVA[2]
Animal Isolates			
ASIL K7978 / Namibia	1 / 16 (6)	6	
Ames / USA	2 / 16 (13)	4	
ASIL K8091 / Norway	2 / 16 (13)	5	A1
ASIL K9729 / Turkey	2 / 16 (13)	4	
ASIL K1938 / Indonesia	2 / 16 (13)	3	A3
Texas-2 / USA	3 / 16 (19)	4	
BA1031 / South Africa	4 / 16 (25)	4	
BA1018 / South Africa	5 / 16 (31)	4	
ASIL K3519 / Tanzania	5 / 16 (31)	4	
ASIL K4241 / Italy	6 / 16 (38)	2	A1
ASIL K1963 / Canada	6 / 16 (38)	4	
ASIL K1671 / Norway	7 / 16 (44)	3	B1
ASlL K4849 / Mozambique	7 / 16 (44)	2	B1
ASIL K6093 / Croatia	7 / 16 (44)	3	
ASIL K0778 / Canada	8 / 16 (50)	4	
ASIL K2087 / USA	8 / 16 (50)	4	
ASIL K7282 / Germany	9 / 16 (56)	3	
BA1007 / USA	9 / 16 (56)	4	A1
BA1002 / Vollum 1B	9 / 16 (56)	2	
ASIL K6286 / Canada	12 / 16 (76)	4	
BA0018 / Canada	16 / 16 (100)	3	A1

[1] Variable number tandem repeat (VNTR) category for *vrr*A

[2] Multiple-locus VNTR analysis genotype



Keim et al. 2000 J. Bacteriol. 182(10)2928-2936

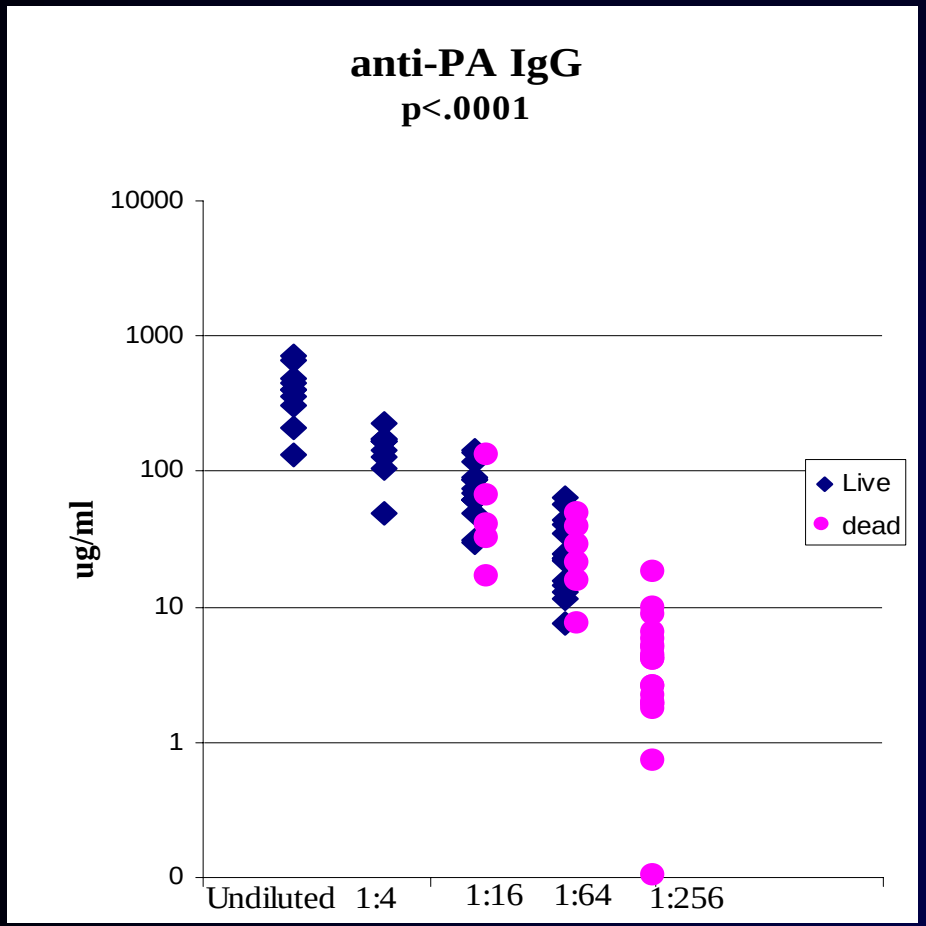
Summary and Conclusions

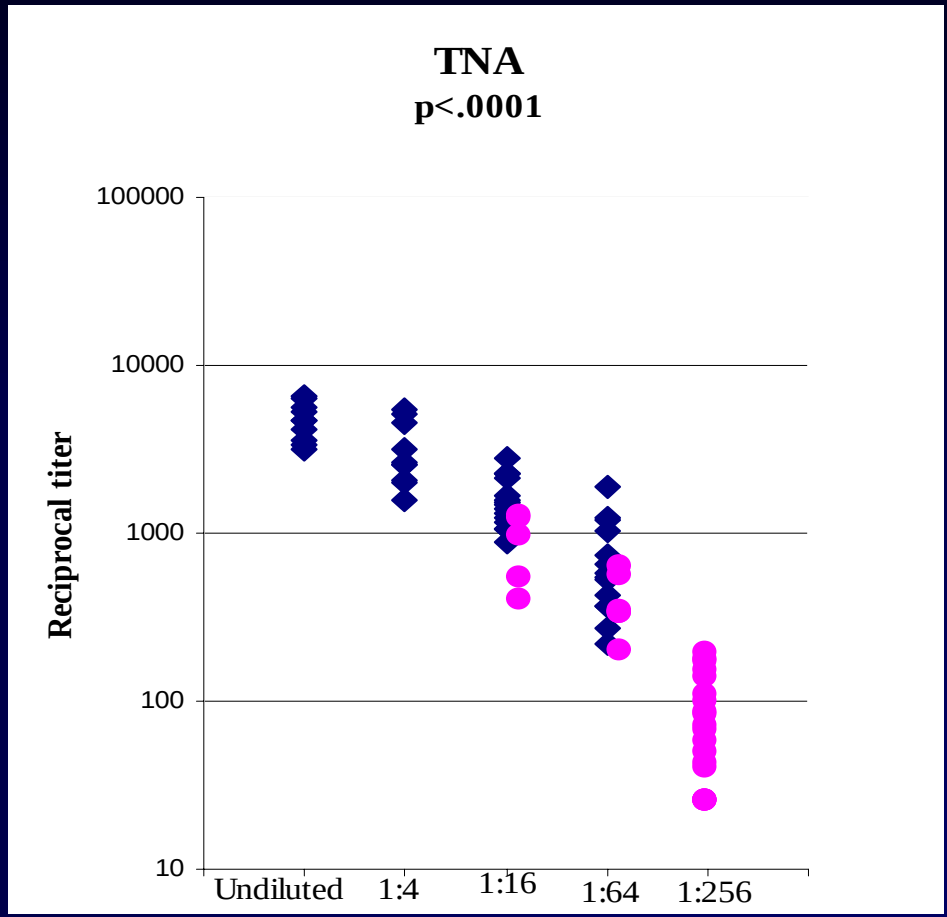
- Guinea pigs vary in the effectiveness of AVA when tested against a geographically diverse group of *B. anthracis* isolates
- There is no relationship between virulence in AVA vaccinated guinea pigs and current genotype analysis of *B. anthracis* isolates
- Both rabbits and rhesus macaques vaccinated 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)

Interval between First Two Doses (wks)	No. of People	% with Titer >1:100	Geometric Mean Titer	p-value
2	22	95%	450	
3	19	100%	1225	0.0090
4	12	100%	1860	0.0015

[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

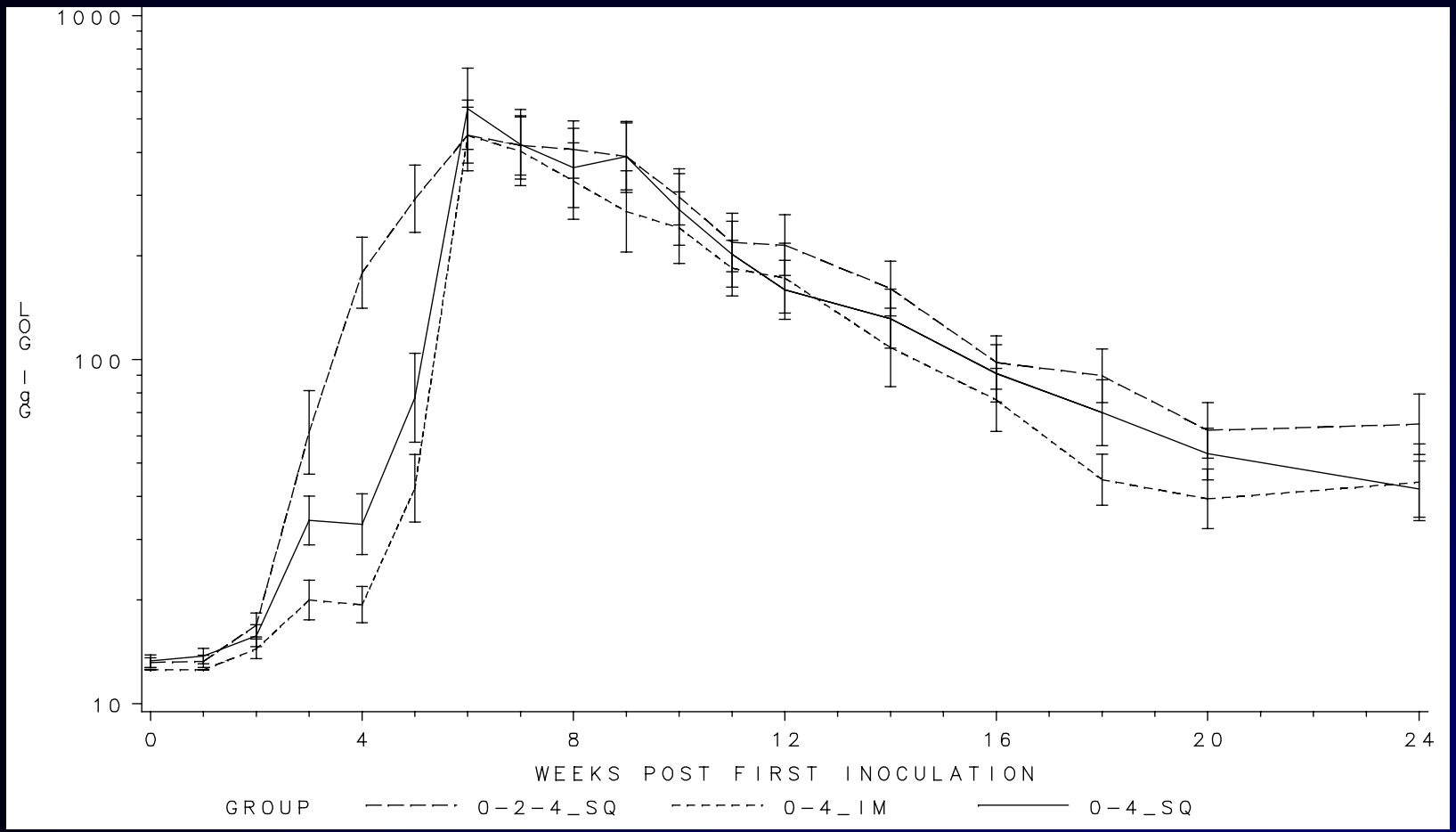
- Determine the best two dose schedule for anthrax vaccine administration
- Determine the better route (IM vs SQ) of anthrax vaccine administration
- Collect safety data

Study Design

Group	Vaccination Schedule	SQ	IM
0	0 week	+	+
0-2	0-2 weeks	+	+
0-4	0-4 weeks	+	+
0-2-4	0-2-4 weeks, 6-12-18 months	+	-

- 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

GUINEA PIGS		
	rPA	21/60 survivors (35%)
	Controls	0/10 survivors
RABBITS		
	rPA	25/30 survivors (83%)
	Controls	0/10 survivors
NON-HUMAN PRIMATES		
	rPA	64/68 survivors (94%)
	Controls	0/11 survivors

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

P. Pittman,G. Kim-Ahn, D. Pifat, K. Coonan, P. Gibbs, S. Little, J. Pace-Templeton, R. Myers, G. Parker. J. Mangiafico, T. Cannon

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

B. Ivins, P. Fellows, M. Linscott, S. Leppla, S. Little, D. Waag, L. Pitt, S. Welkos, J. Farchaus, G. Benner. CpG: D. Klinman

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

G. Andrews, B. Powell, W. Ribot, B. Ivins, S. Little, L. Pitt, P. Fellows, J. Farchaus, P. Worsham, G. Anderson