

WHY INFECTIOUS DISEASES

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Public Health Works -
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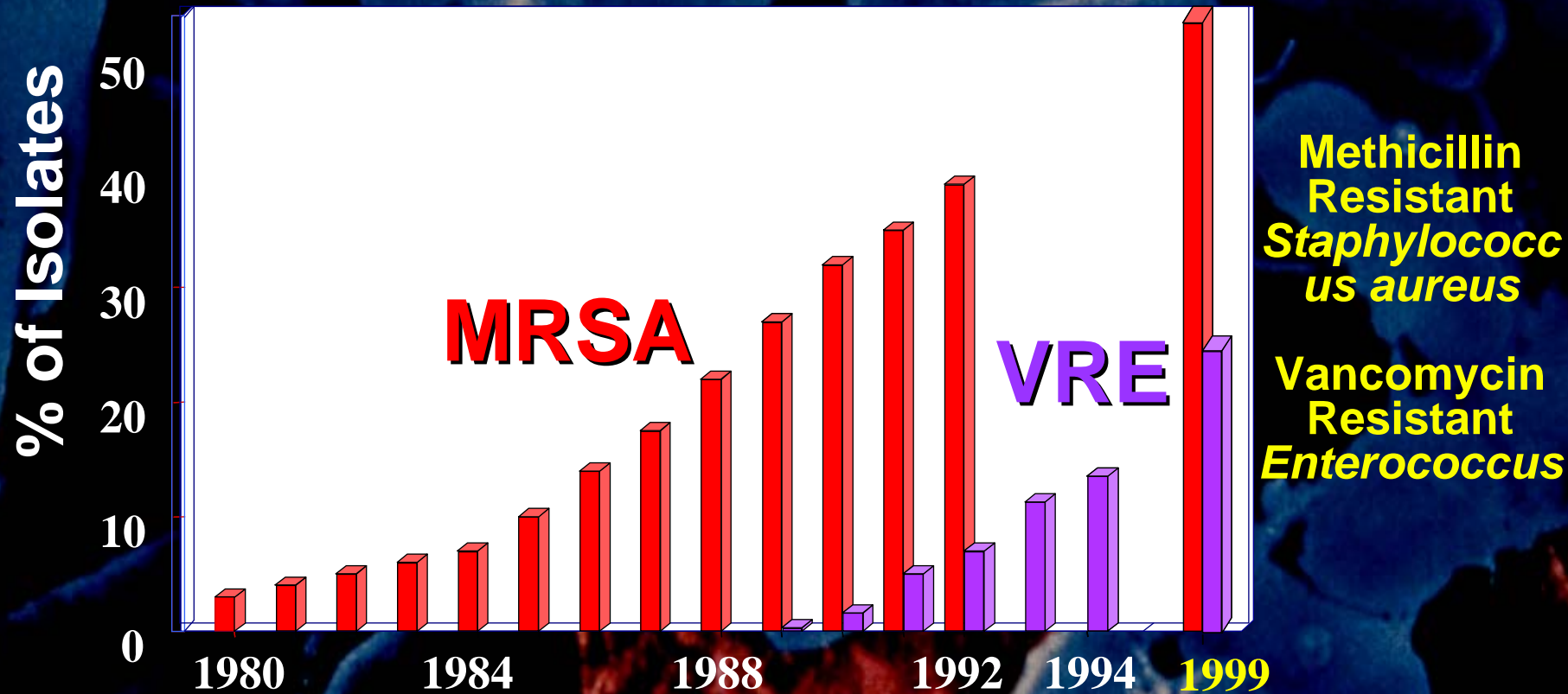
Global Mortality (WHO – 1999)

- 54 million annually
- 16.5 million due to infectious diseases
- 12 million due to cardiovascular disease
 - Chlamydia
- 5 million due to cancer
 - Helicobacter, HPV-16 & 18, EBV

Challenges

- Development of Drug resistant pathogens**
- Alarming increase in nosocomial infections**
- Aging population**
- Upward spiraling health costs**
- Immunosuppressed patients**
- New emerging diseases**
- Bioterrorism**

The Need for New Antimicrobial Discovery



No blockbuster antibiotics for 20 years or so.
Since 2001 several companies have stopped
all antibiotic development

CAUSES OF EMERGING DISEASES

1. Evolution

- Mutation
- Recombination/re-assortment

2. Host Modification

- Behavior (drug use, STD's, daycare)
- Transplantation (immunosuppression)
- International travel

3. Recognition

- Diagnostics

4. Increased contact with vectors

- Invade ecological niches

VACCINES

“Vaccination is one of the most cost-effective approaches for the management of infectious disease”

VACCINES/DISEASE ERADICATION

- Small pox – 1980
- Polio – WHO target 2005
- Measles – WHO target 2010
- 30 new /re-emerging diseases in last 30 years
- Zoonotic infections 50% - 75%

TYPES OF VACCINES

1. CONVENTIONAL

- Live
- Inactivated

2. GENETICALLY ENGINEERED

- Live
- Live chimeric
- Live replication defective
- Subunit
 - Monovalent vs. chimeric
 - Peptide
 - Plant based vaccines
- Polynucleotide
- Plant based

REQUIREMENTS FOR SUBUNIT VACCINES

- Knowledge of antigens involved in inducing protection
- Knowledge of specific responses involved in protection
 - Antibody vs. CMI
 - Mucosal vs. Systemic Immunity
 - Sterile vs. Protective Immunity

Technologies for Subunit Antigen Identification

1. Conventional

- Brute force
- Comparative
- Immunological

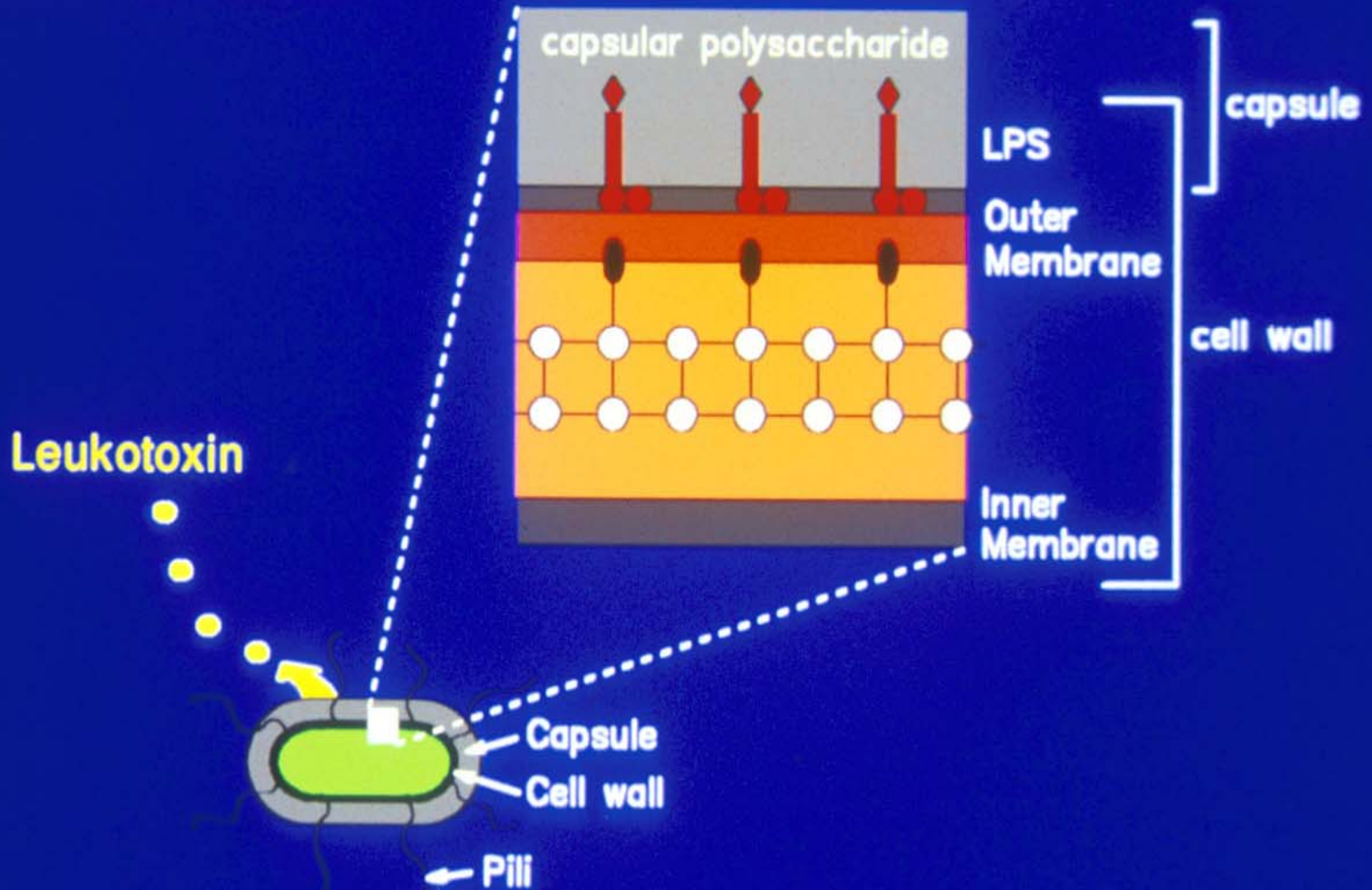
2. Genomics

- Comparative
- Colonization

ANTIGENS OF P. haemolytica

Secreted Antigens

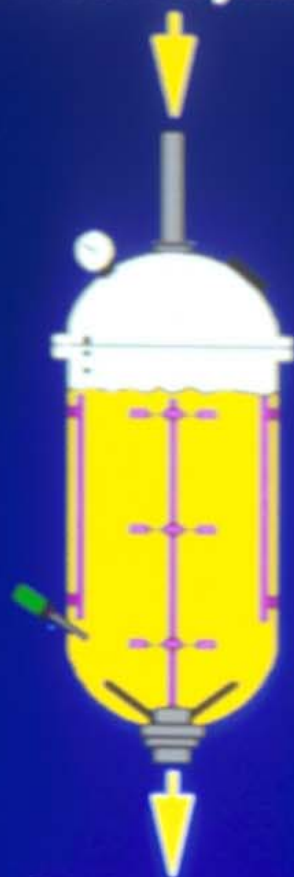
Structural Antigens



BACTERIAL VACCINE MANUFACTURING

IMPROVED MANUFACTURING EFFICIENCY THROUGH GENETIC ENGINEERING

P. haemolytica



10^9

leukotoxin
1X
1X

E. coli containing
leukotoxin gene
from *P. haemolytica*

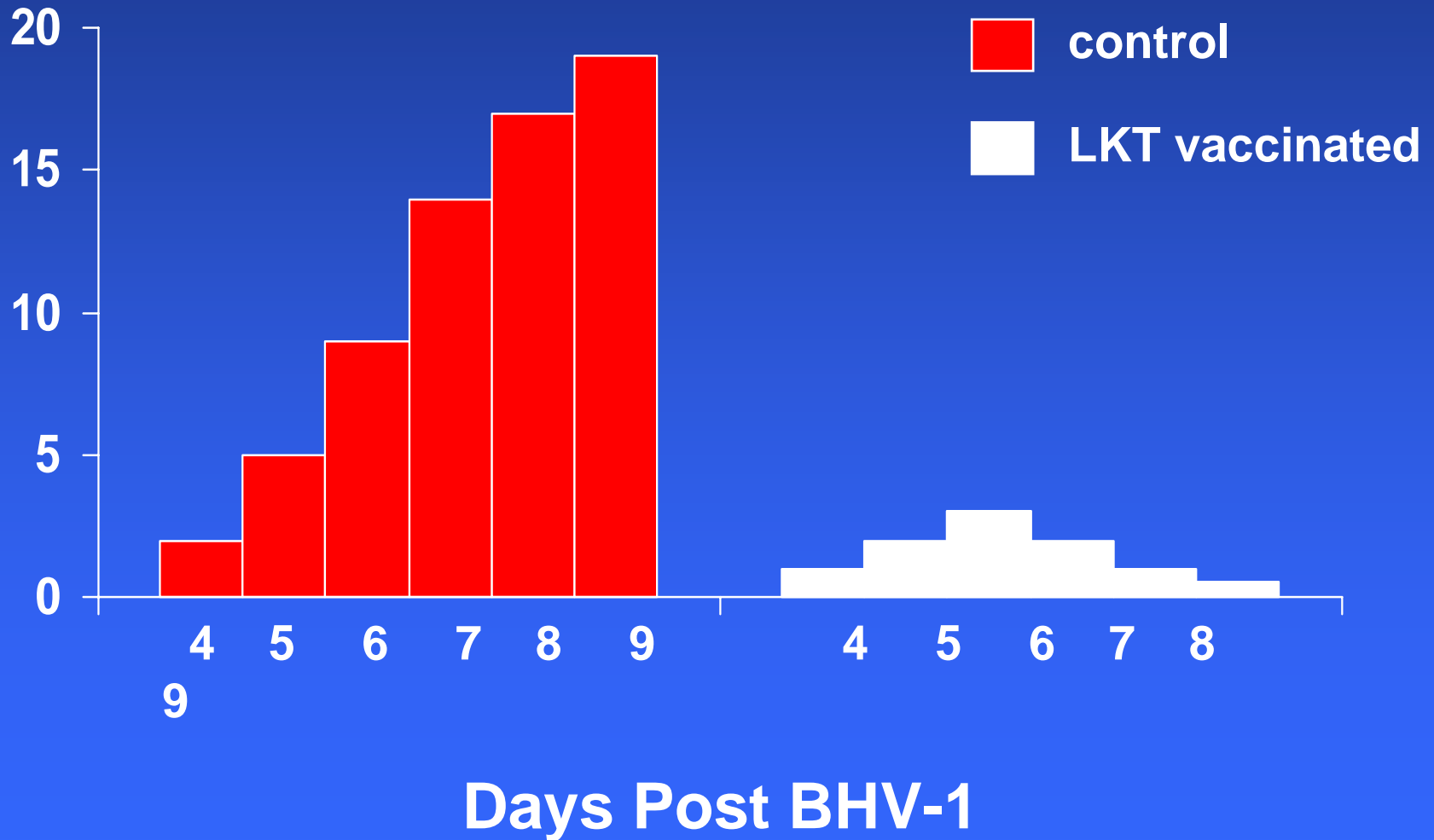


10^{10}

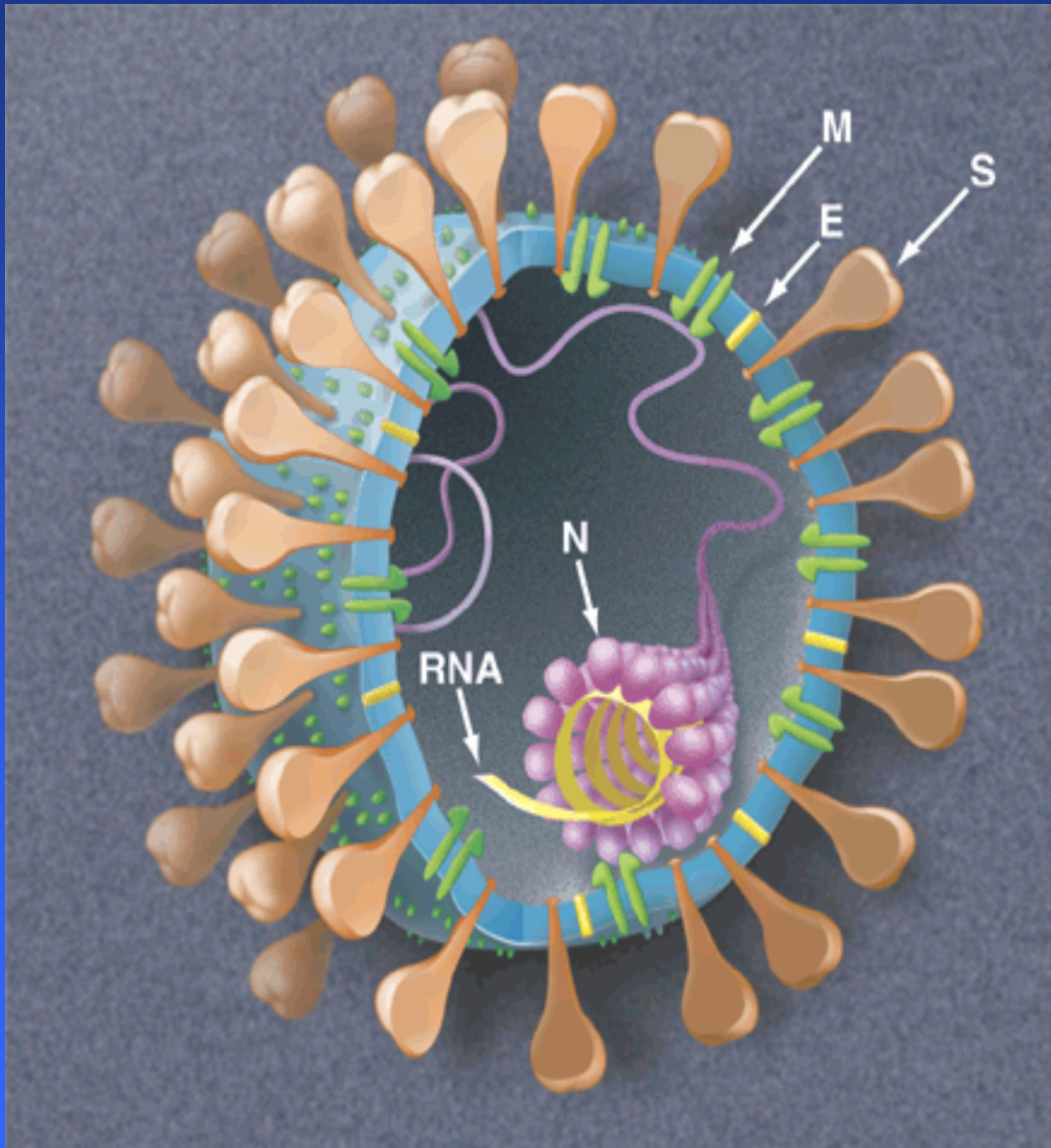
rDNA leukotoxoid
100 - 1,000X
1,000 - 10,000X

YIELD
Per Cell
Total

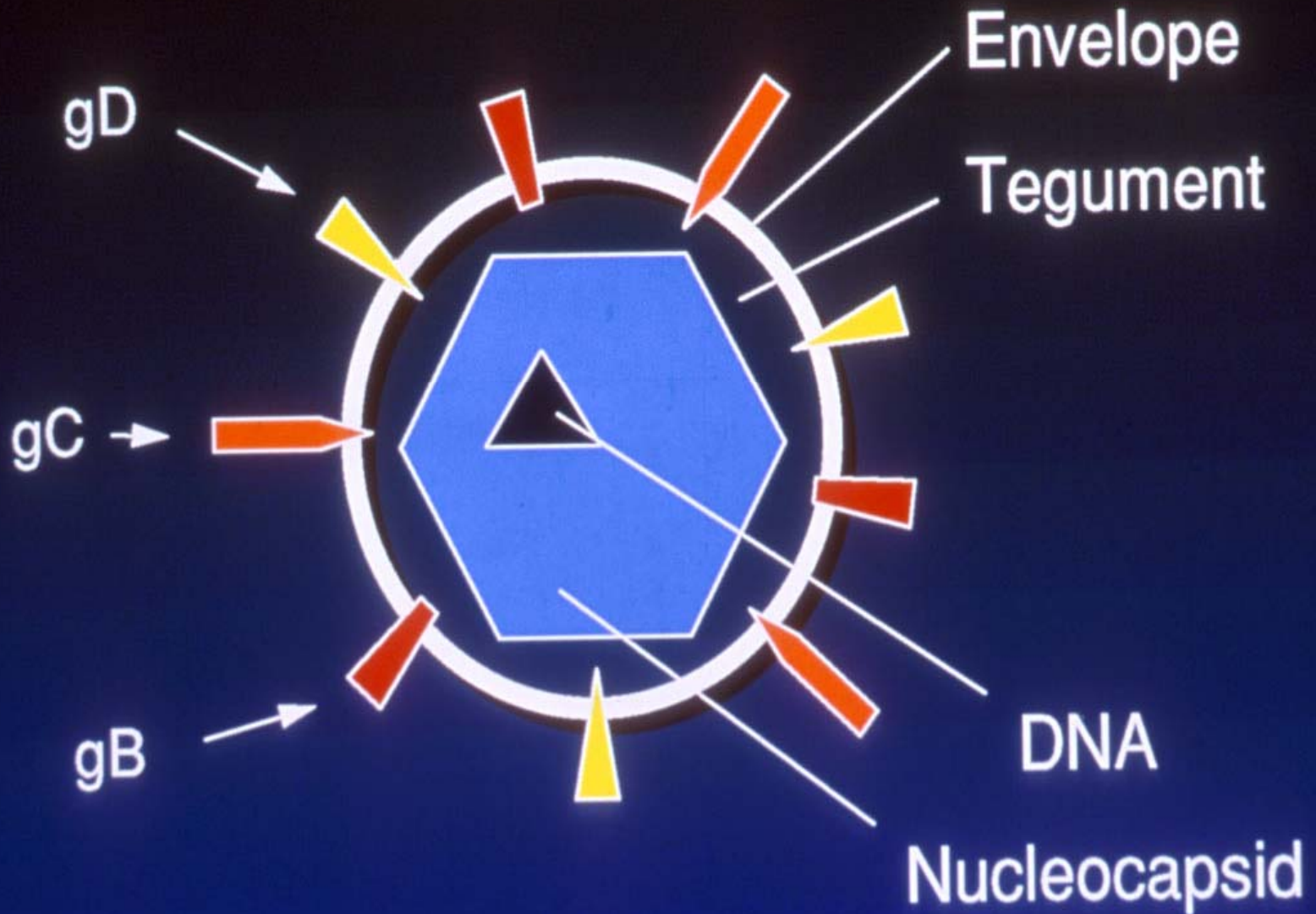
Leukotoxin Vaccination Mean Clinical Score



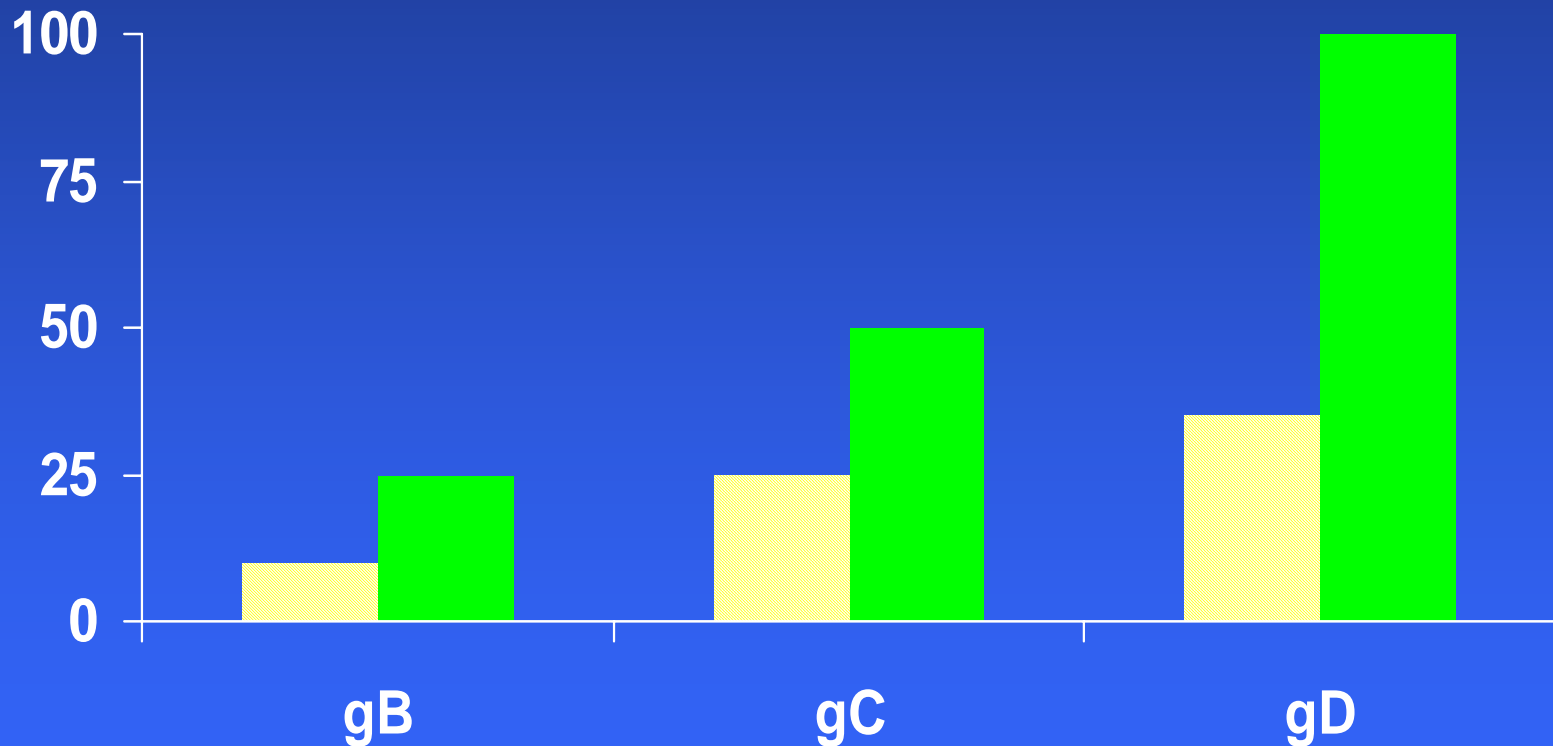
SARS Coronavirus



BHV-1 virion

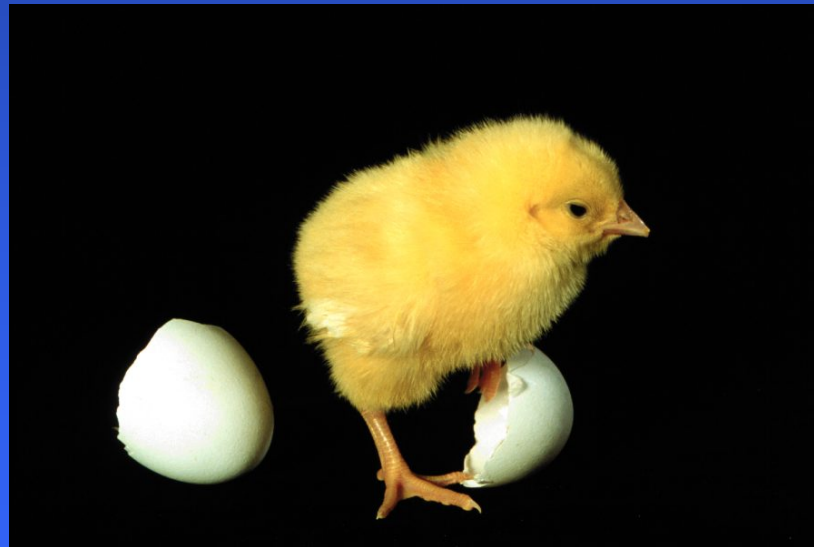


Induction of Neutralizing Antibody by BHV-1 Glycoproteins

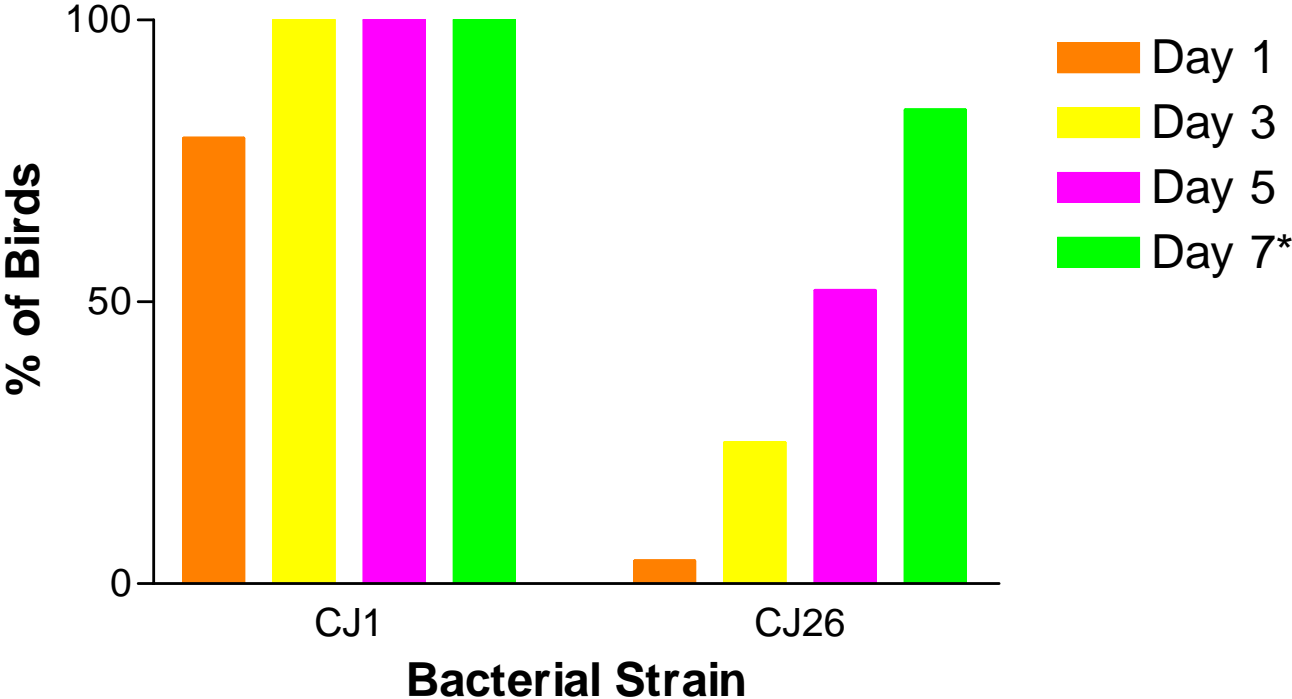


Genomics in Antigen Identification

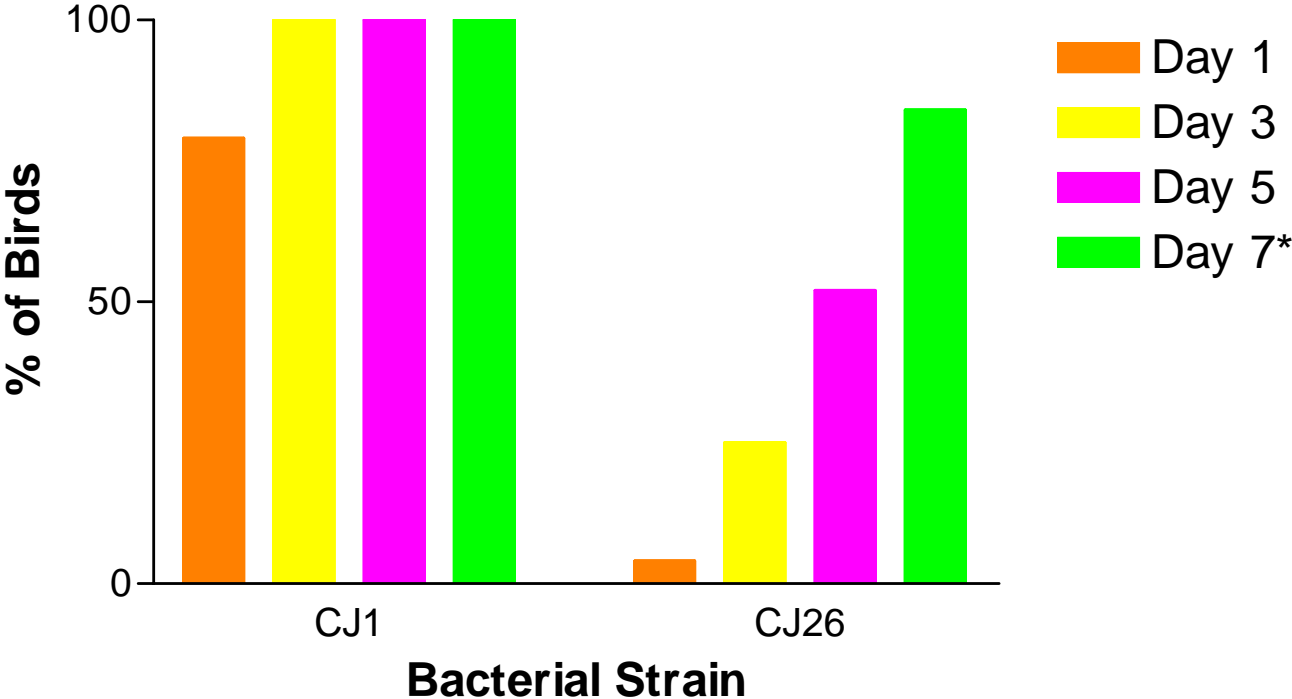
Comparison of Two Lineages of NCTC11168



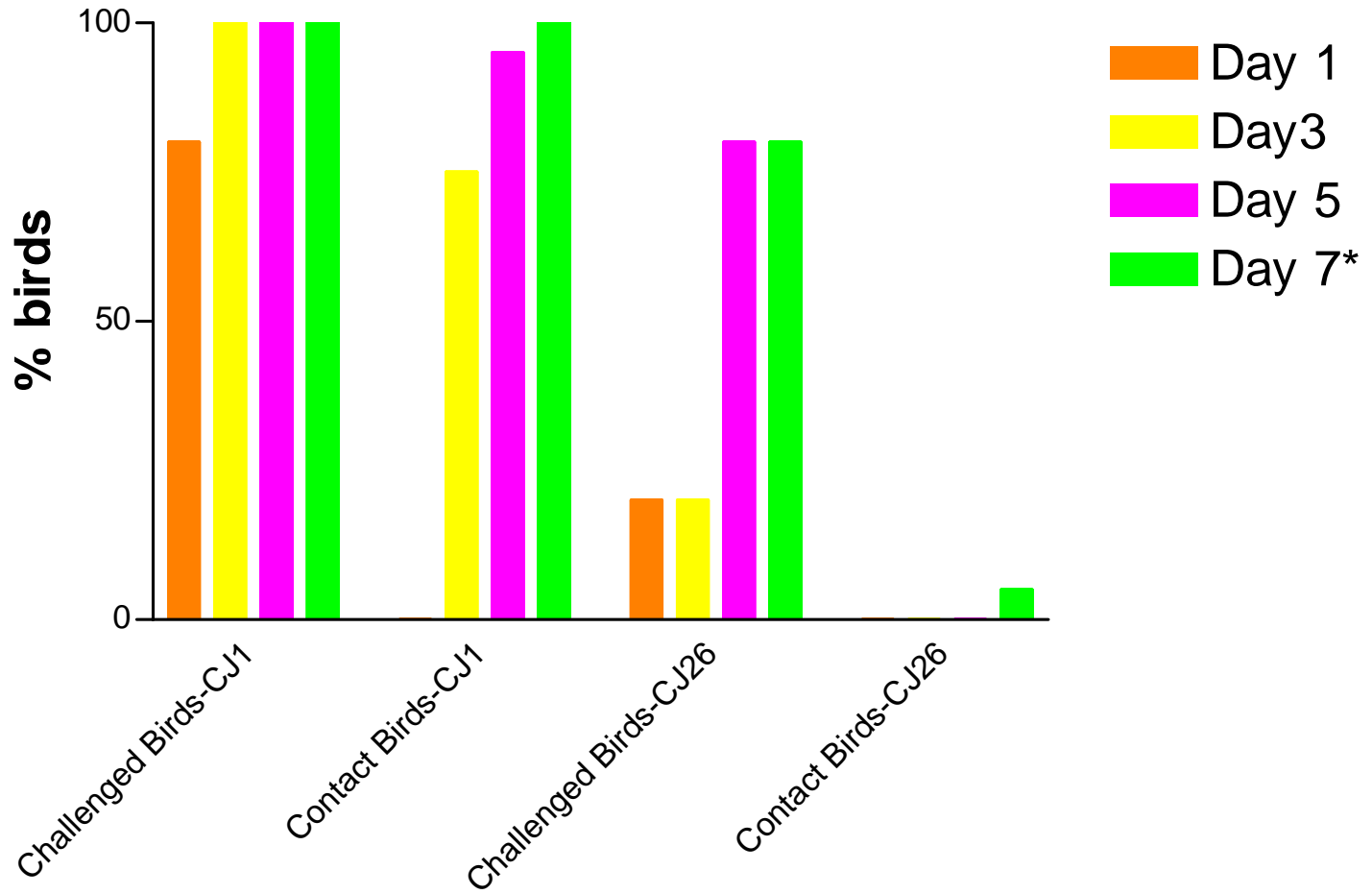
Comparison of Colonization by CJ1 and CJ26



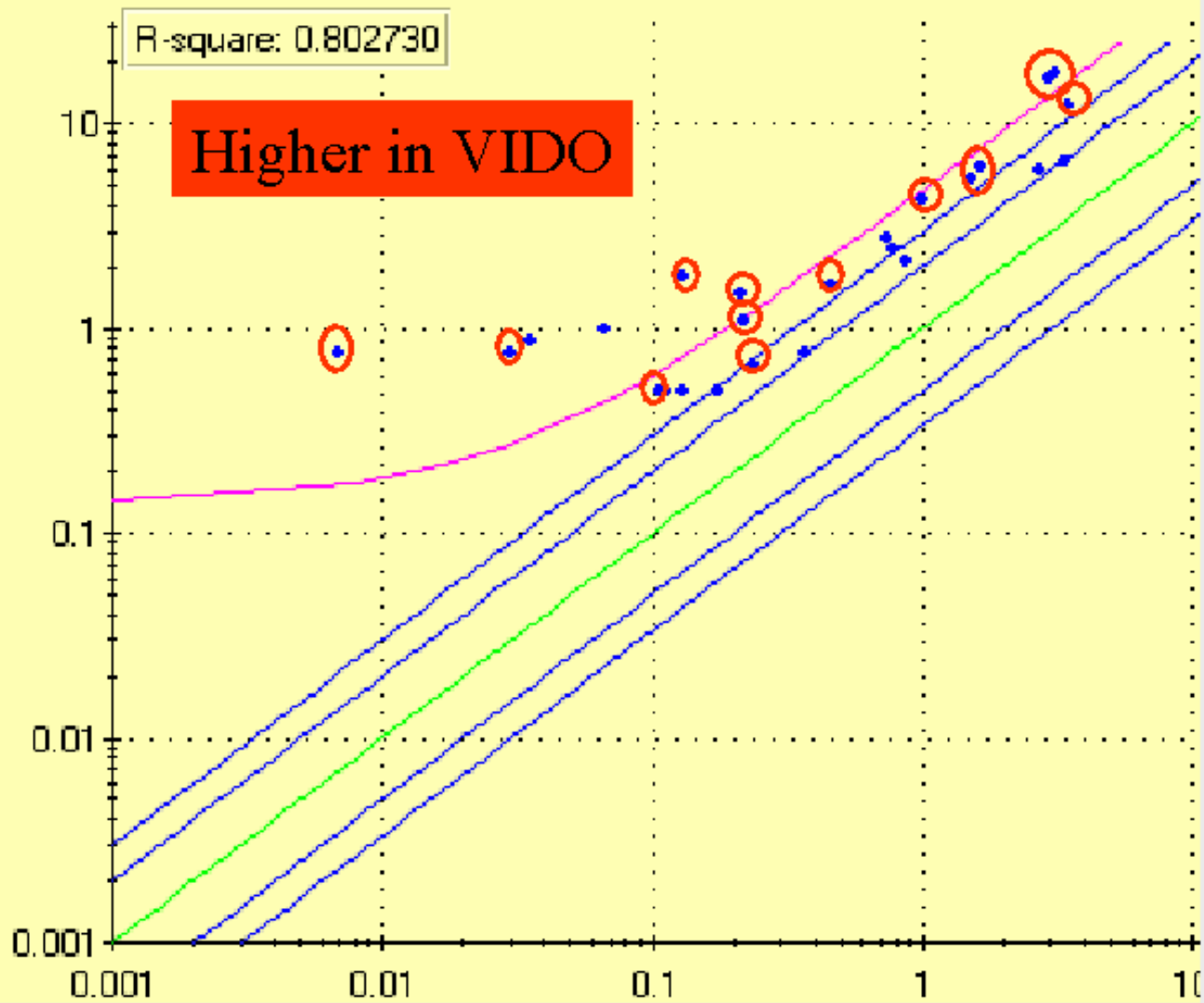
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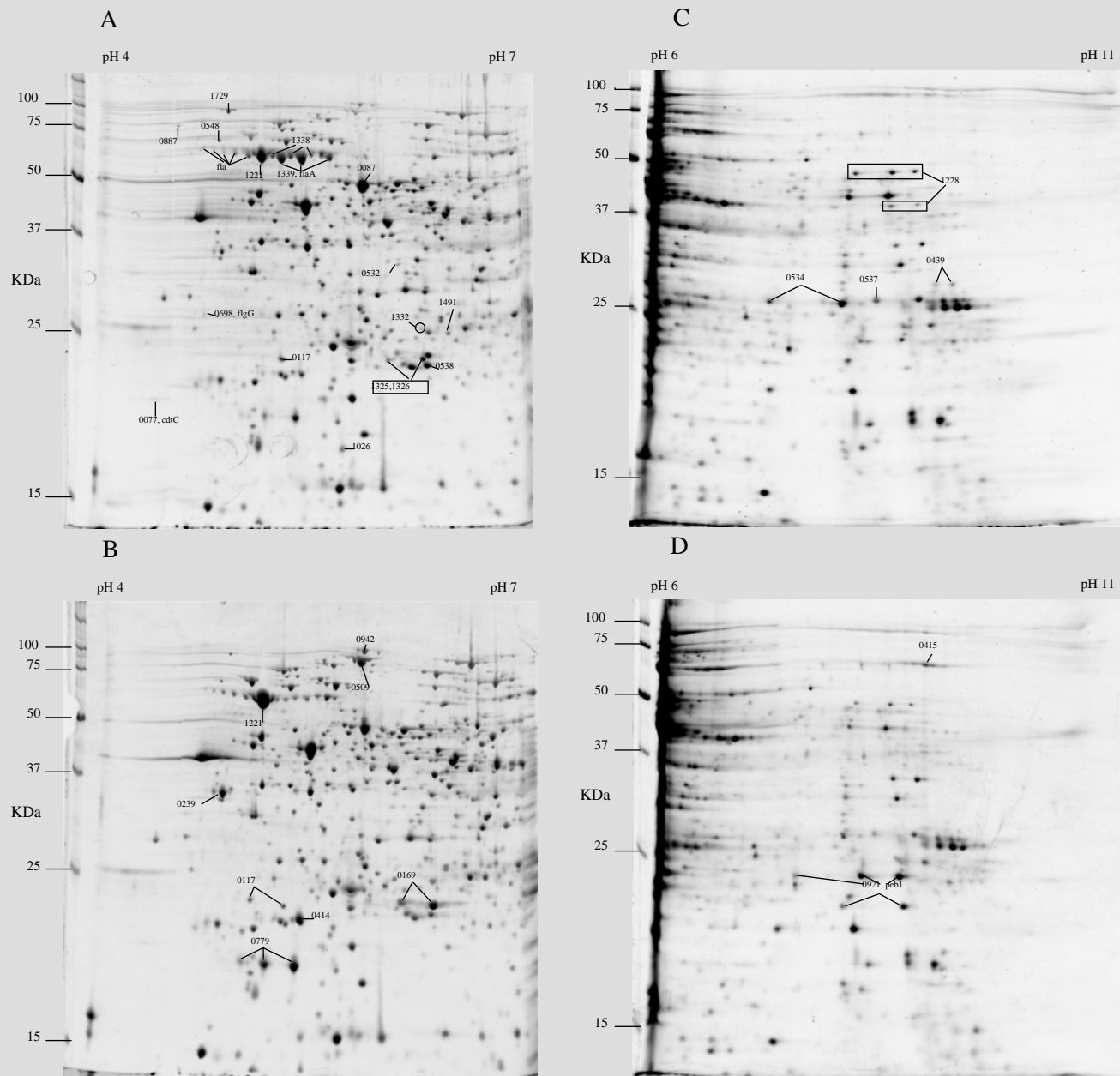
Horizontal Transfer (AS02-229)



A

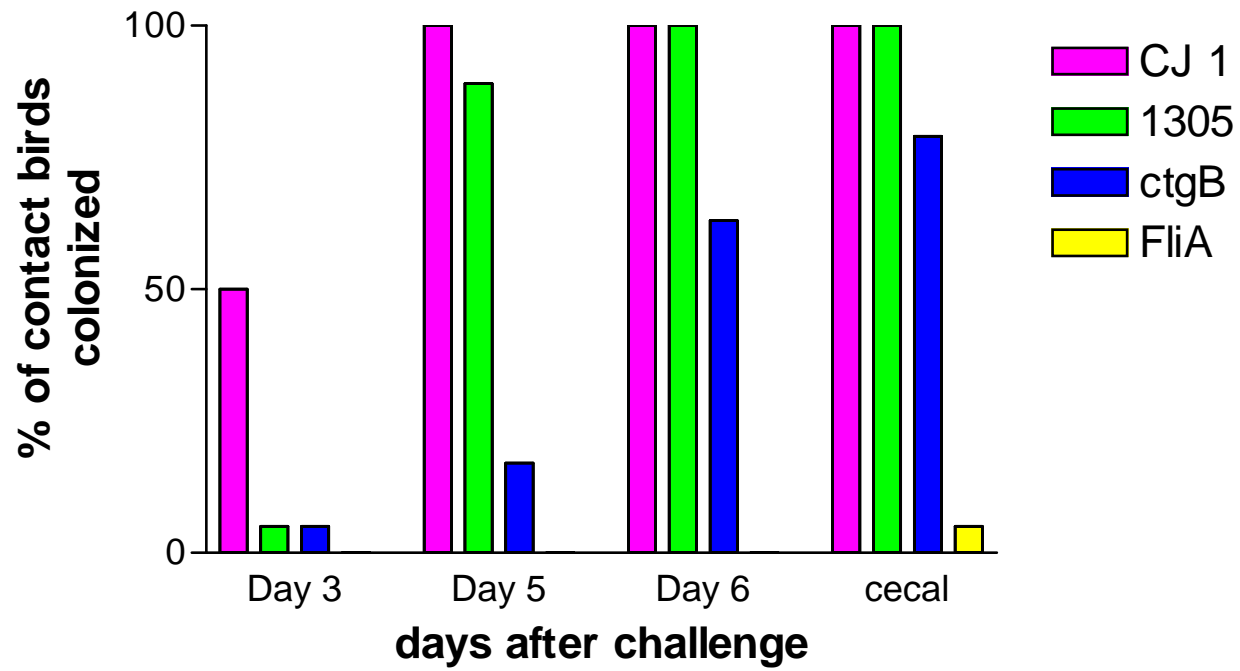


Proteome profiling: VIDO vs NRC



Genes of CJ1 with a higher level of transcription that have been confirmed to be up-regulated by proteomic

| | |
|--|---|
| cdtC cj0077c cytolethal distending toxin | fliD cj0548 flagella protein |
| cj1026c putative lipoprotein | flaD cj0887c putative flagellin |
| cj1325 hypothetical protein | flaB cj1338c flagellin |
| cj1326 hypothetical protein | flaA cj1339c flagellin |
| htrA cj1228c serine protease | flaE2 cj1729 flagellar hook protein |
| | flgG cj0698 flagellar basal body |
| | ptmA cj1332 oxidoreductase flagellin modification |



VACCINE FORMULATION & DELIVERY

- **FORMULATION**
 - Liposomes
 - Alginate/ PLG microspheres
 - Polyphosphazenes
 - Plant-based formulations
 - DNA vaccines
 - Vectors (viral)
- **DELIVERY**
 - Intranasal
 - Oral
 - Transcutaneous
 - Fetal immunization

FORMULATION

1. Adjuvants

- Alum
- MF 59

2. Immune Modulators

- Shift immune responses
- Cytokines
- CpG

CpG DNA – a “Danger Signal”

| <u>DNA</u> | <u>Bacterial DNA</u> | <u>Vertebrate</u> |
|---------------------|----------------------|-------------------|
| Immune stimulatory? | Yes | No |
| CG frequency | random (1/16) | suppressed (1/60) |
| C methylation | No | usually |

Thus, the immune system has evolved the ability to recognize unmethylated CpG sequences as a “danger signal” indicating infection.

Bacterial genomes are too large to permit mutations to remove all CpG, but many viruses with small genomes have deleted CpG to circumvent our immune responses.

What is a CpG Motif?

$X_1 X_2 \underline{CG} Y_1 Y_2$

$X_1 > \text{purine}$

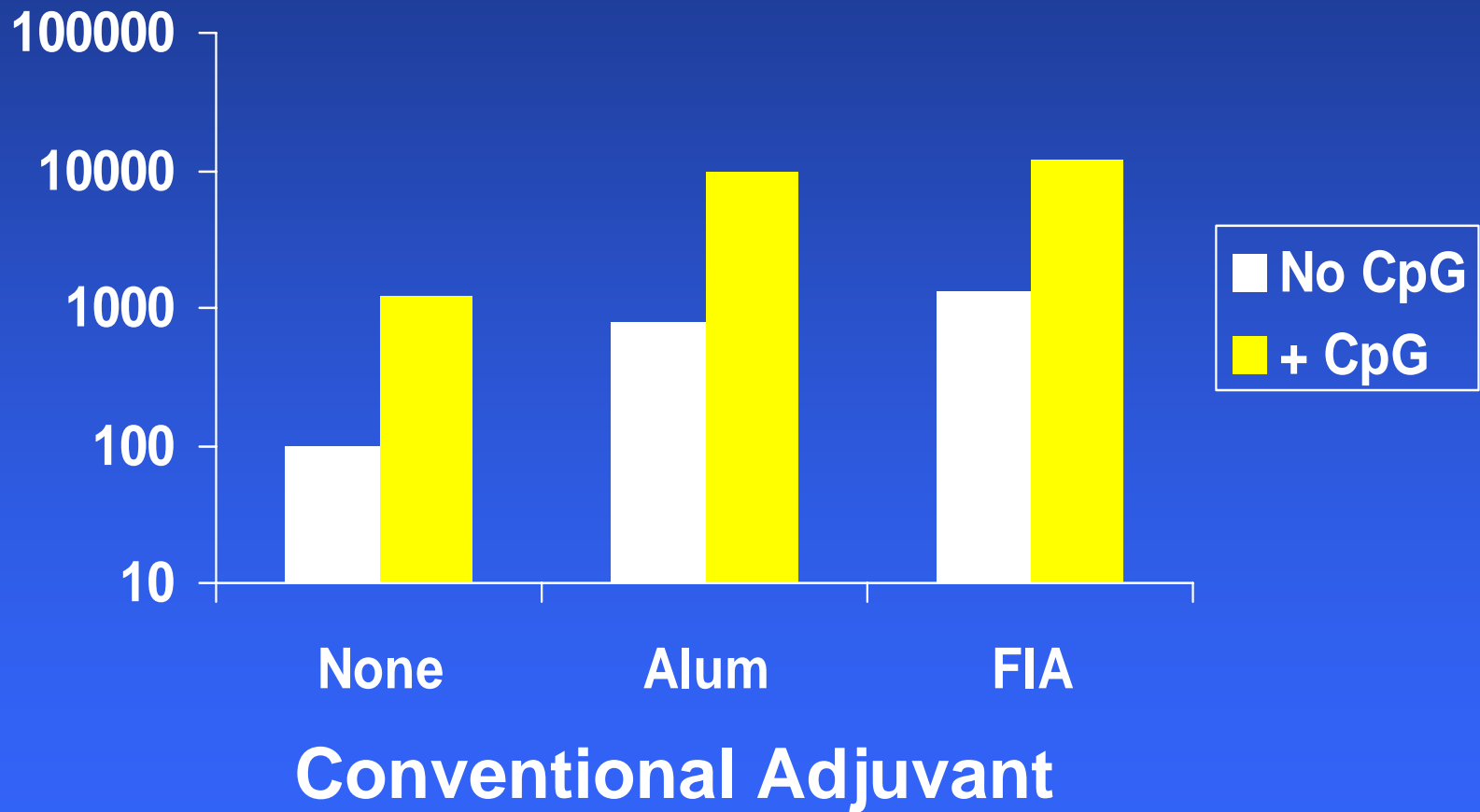
$X_2 = \text{not C}$

$Y_1 = \text{not G (T best)}$

$Y_2 > T$

- Immune effect of CpG motifs determined by:
 - Number, position, and spacing of CpG motifs
 - ODN backbone (native DNA vs. nuclease resistant linkages)
- Species specificity for optimal motifs
- Mouse 1826 - TCCAT GACG TTCCT GACG TT

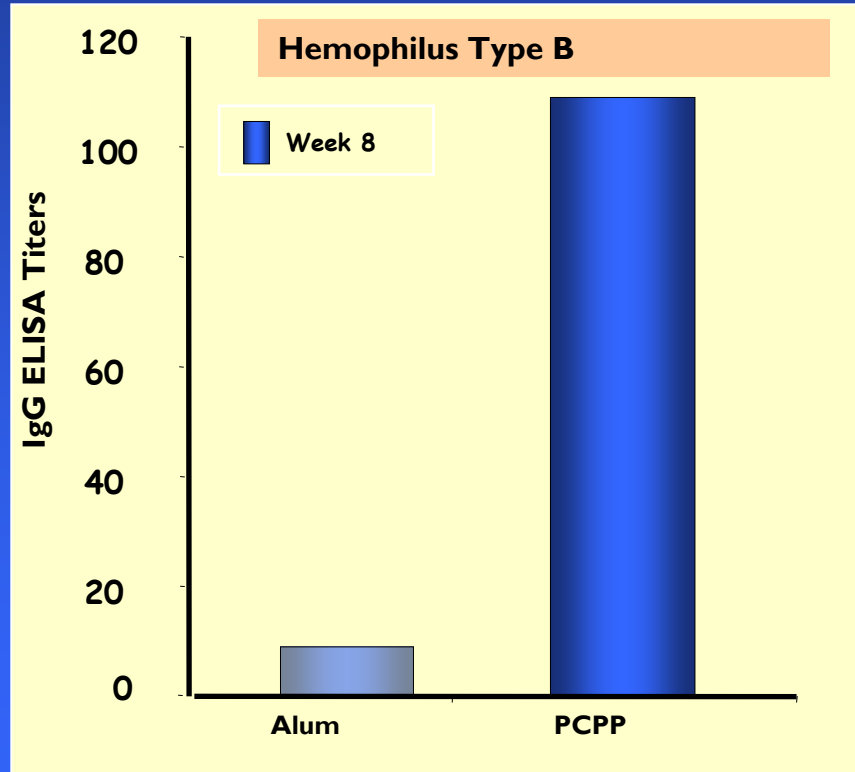
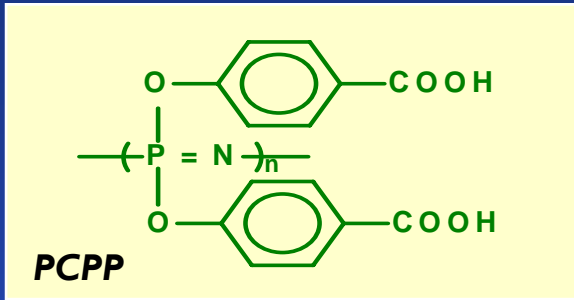
Adjuvant Effect of CpG ODN in Mice



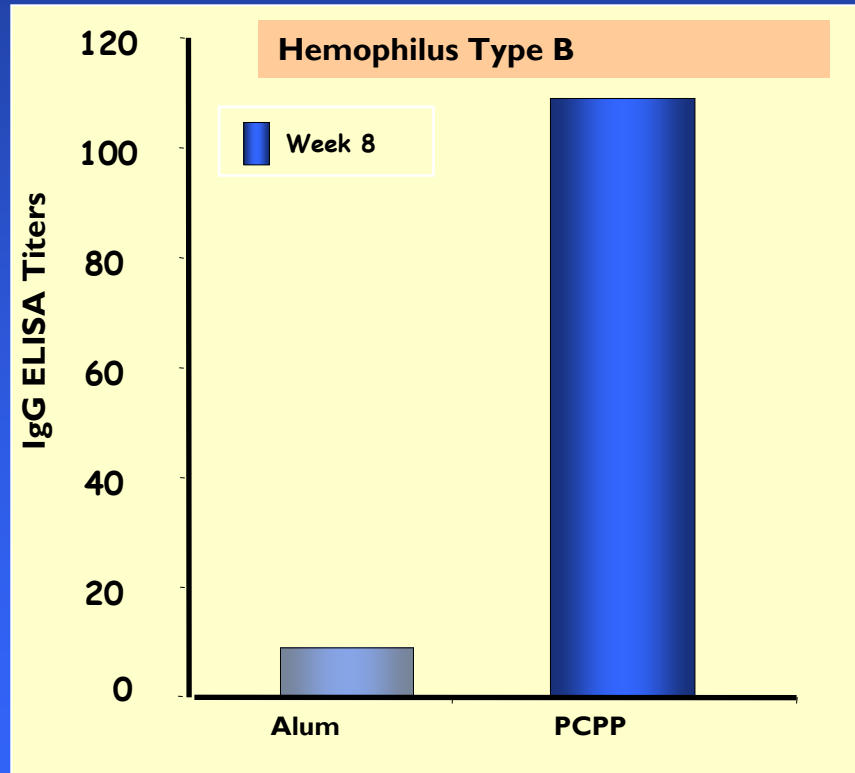
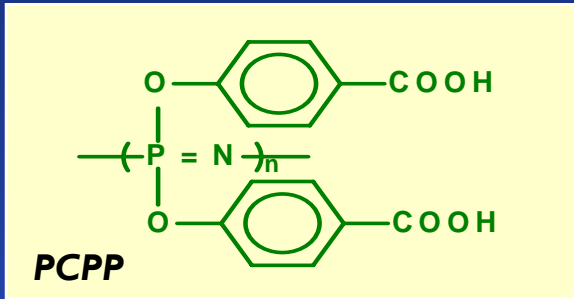
Advantages of CpG as an Adjuvant

- Reduced tissue damage
- Increased immune responses
- More balanced immune response (cellular & humoral)
- Reduced antigenic mass required

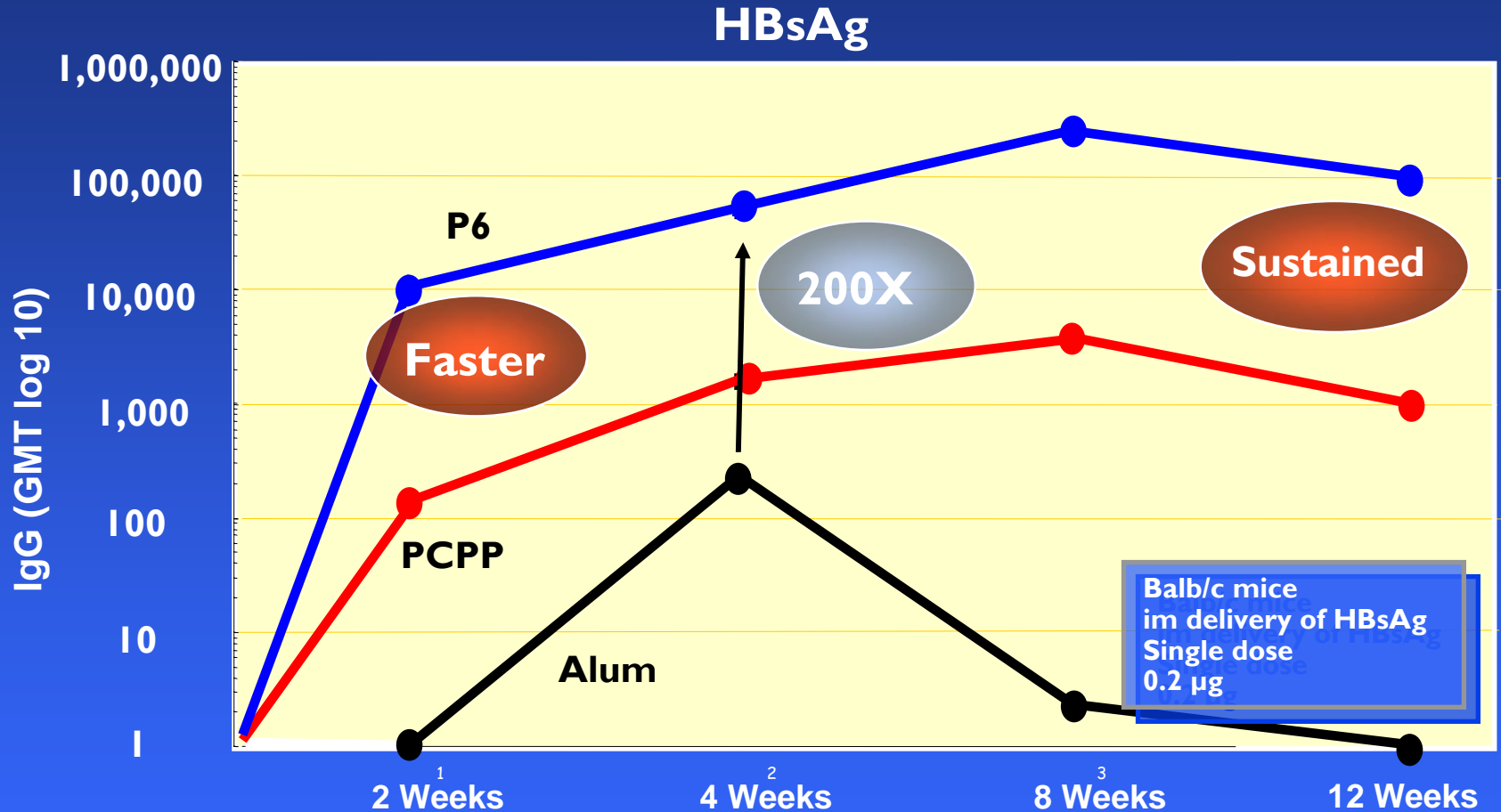
A first generation molecule, PCPP, has a potent and sustained immune response



A first generation molecule, PCPP, has a potent and sustained immune response



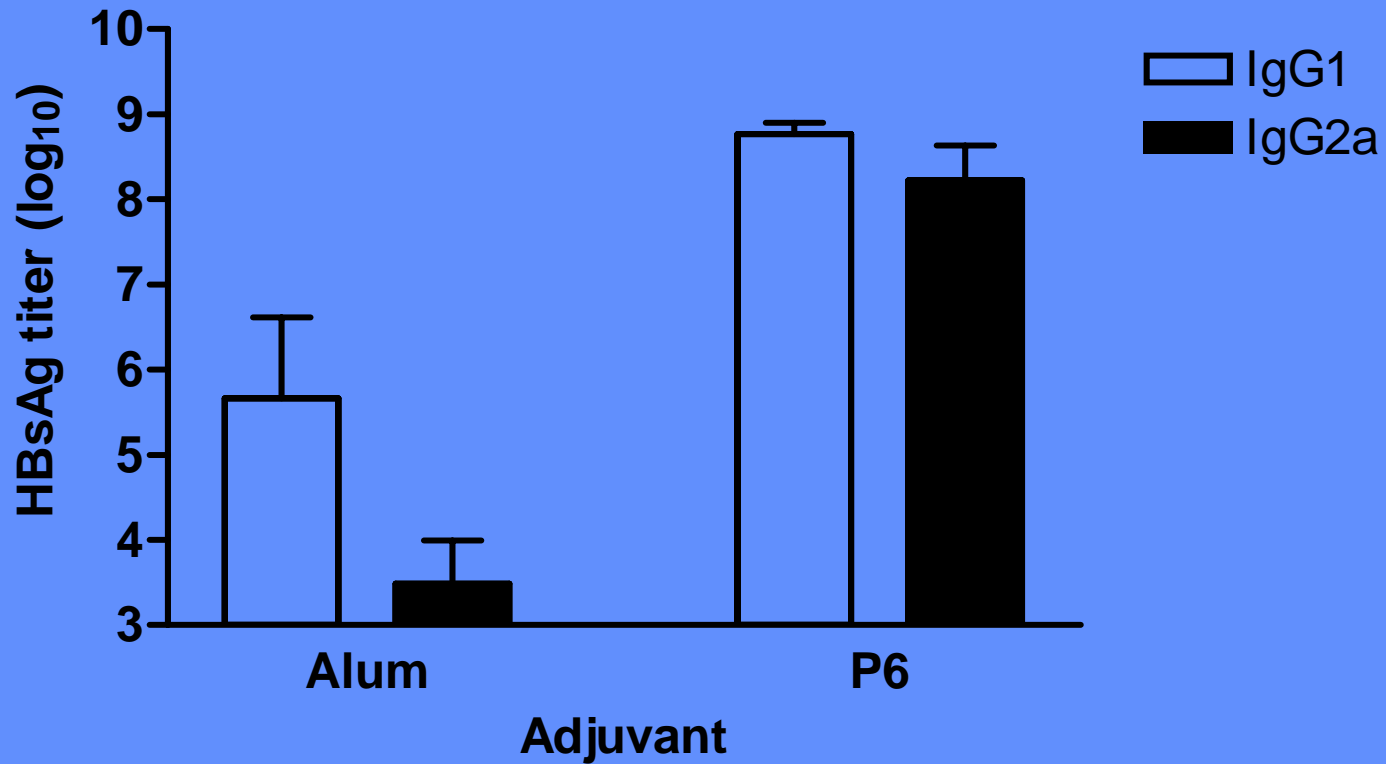
Second Generation Molecules: Faster Kinetics and Sustained Response



.....Response continues at 8 weeks

Note: ~3,000 highest with alum vs $1.3 \times (10)^6 = 430x$ with P6

Polyphosphazenes enhance a balanced Th1/Th2 response (12 weeks)



Alternative Vaccine Delivery Systems

Transcutaneous



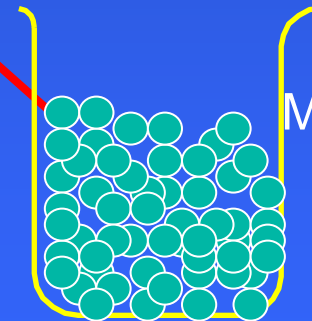
Microneedles



Intranasal



Aerosol Delivery



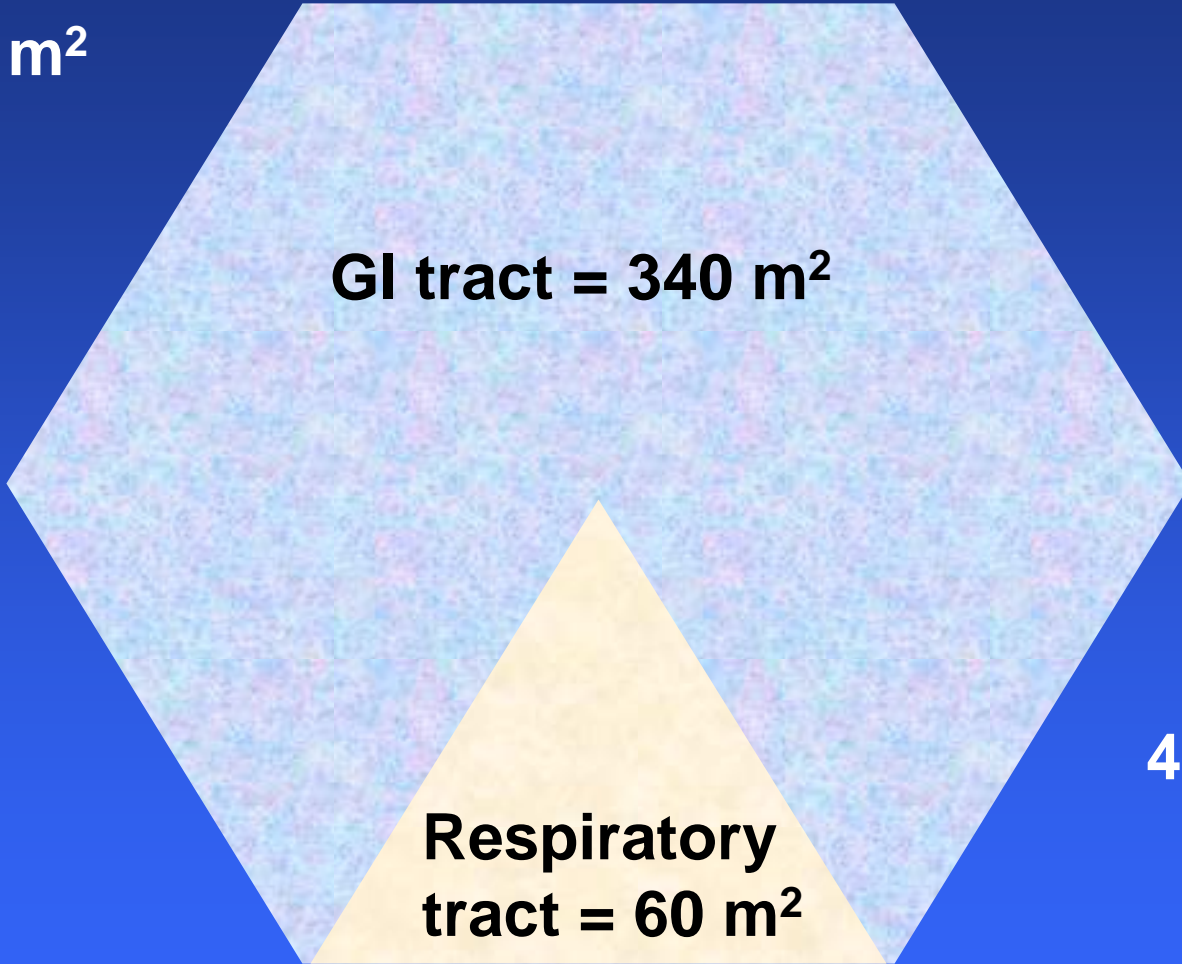
Oral
Microsphere
Carriers

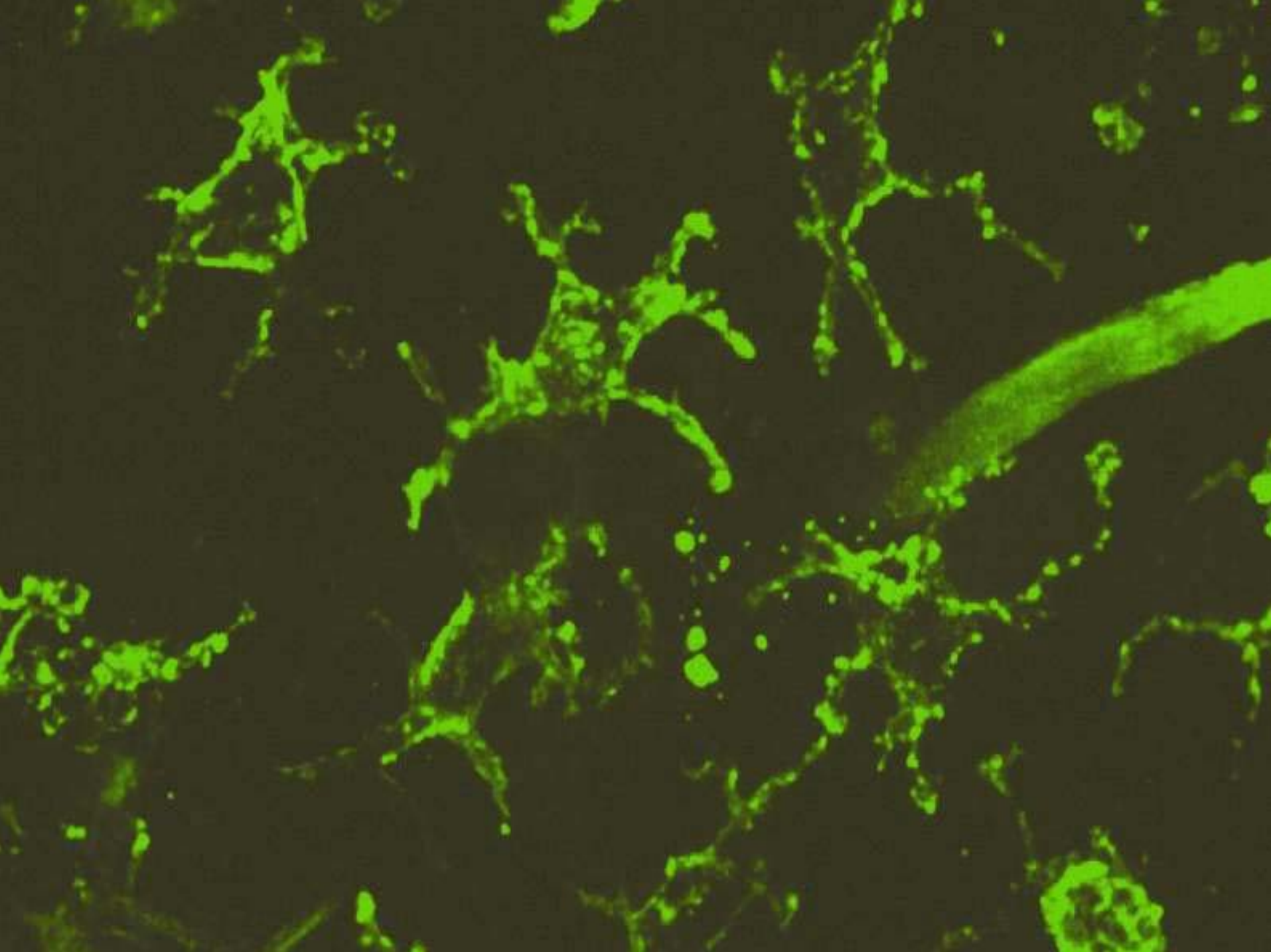
Skin 2 m²

GI tract = 340 m²

**Respiratory
tract = 60 m²**

4304 sq ft



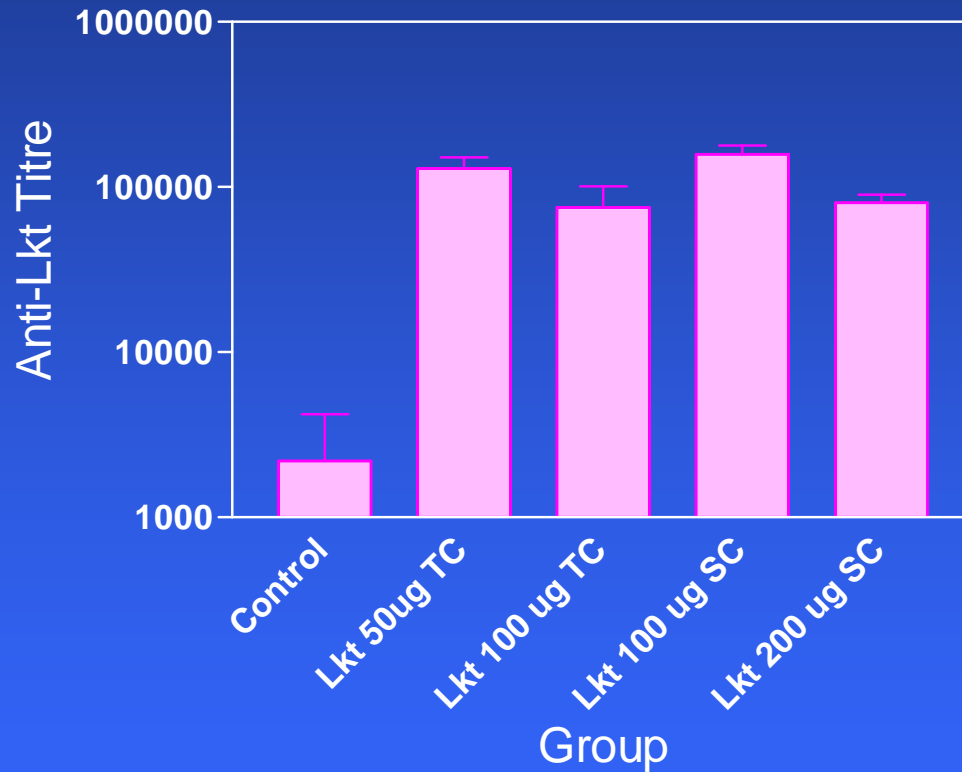




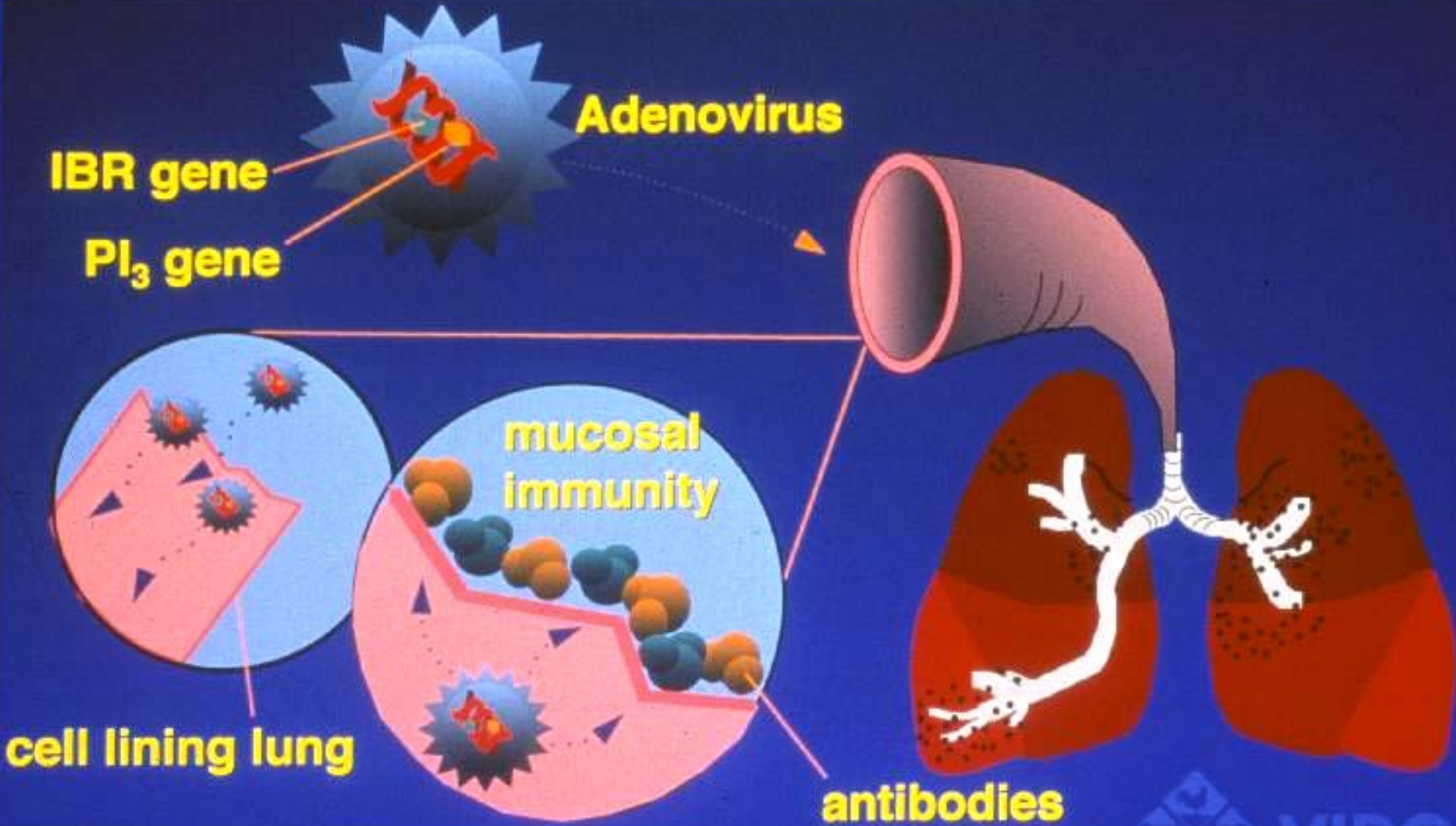


Transdermal Immunization

Serum Anti-LKT IgG



Mucosal Immunity



ROLE OF MUCOSAL ANTIBODIES

- Neutralize virus – prevents attachment
- Interference with transcription
- Enhanced antigen uptake – antigen presentation

STRATEGIES FOR ENHANCING MUCOSAL IMMUNITY

Mucosal adjuvants

- cholera toxin – Ag coupling

Live vectors

- viruses, bacteria

Microparticles

- biodegradable microspheres, VLP, ISCOMs, liposomes

DNA vaccines

Transgenic plants

MIGRATION AND HOMING OF LYMPHOCYTES



Eye
Lachrymal
Gland

Oral Cavity
Salivary
Gland

Milk
Mammary
Gland



Inductive sites

(GALT and
BALT)



Common Mucosal Immune System



Lamina
Propria of
Genitourinary
Tract

Lamina
Propria of
Intestine

Lamina
Propria of
Upper
Respiratory
Tract

THE BIPHASIX™

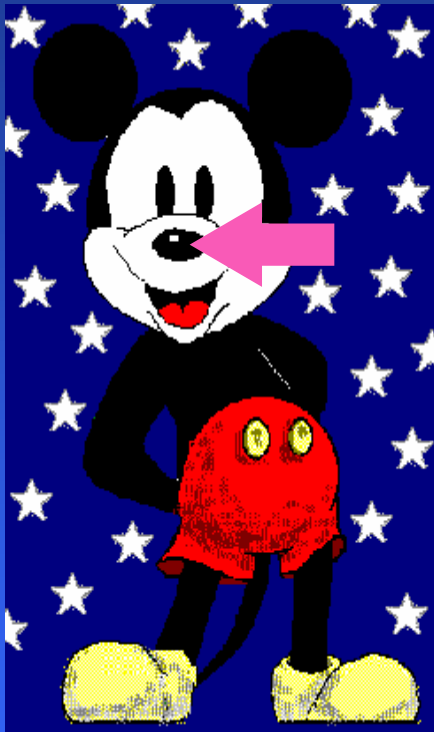


- encapsulate drugs of wide molecular weight range

(up to 100kDa, including peptides, proteins and oligo/polynucleotides)

Experimental Approach

Objective: Induction of mucosal immunity by intranasal delivery of *Y. pestis* whole cell killed vaccine



Boost 3 weeks



Killed 10 days
after boost

- **Mucosal immune response:**

Lung and nasal washes

Lung cells

- **Systemic immune response:**

Serum

Spleen cells

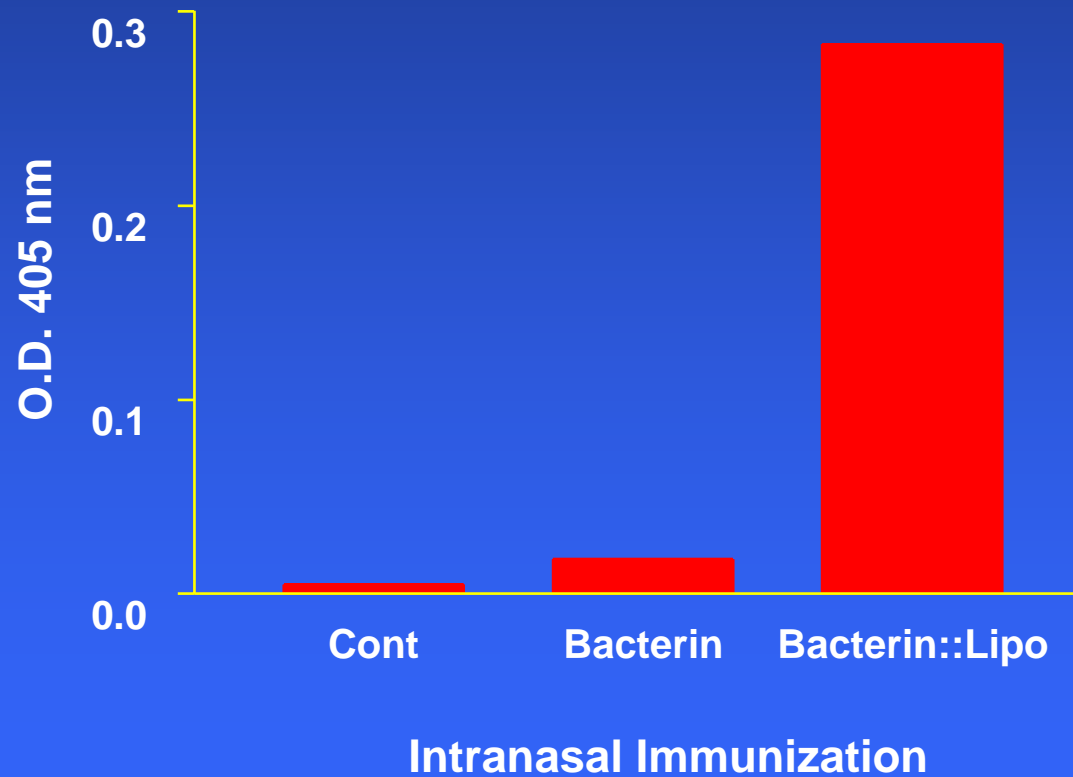
ANTIGENS

PBS

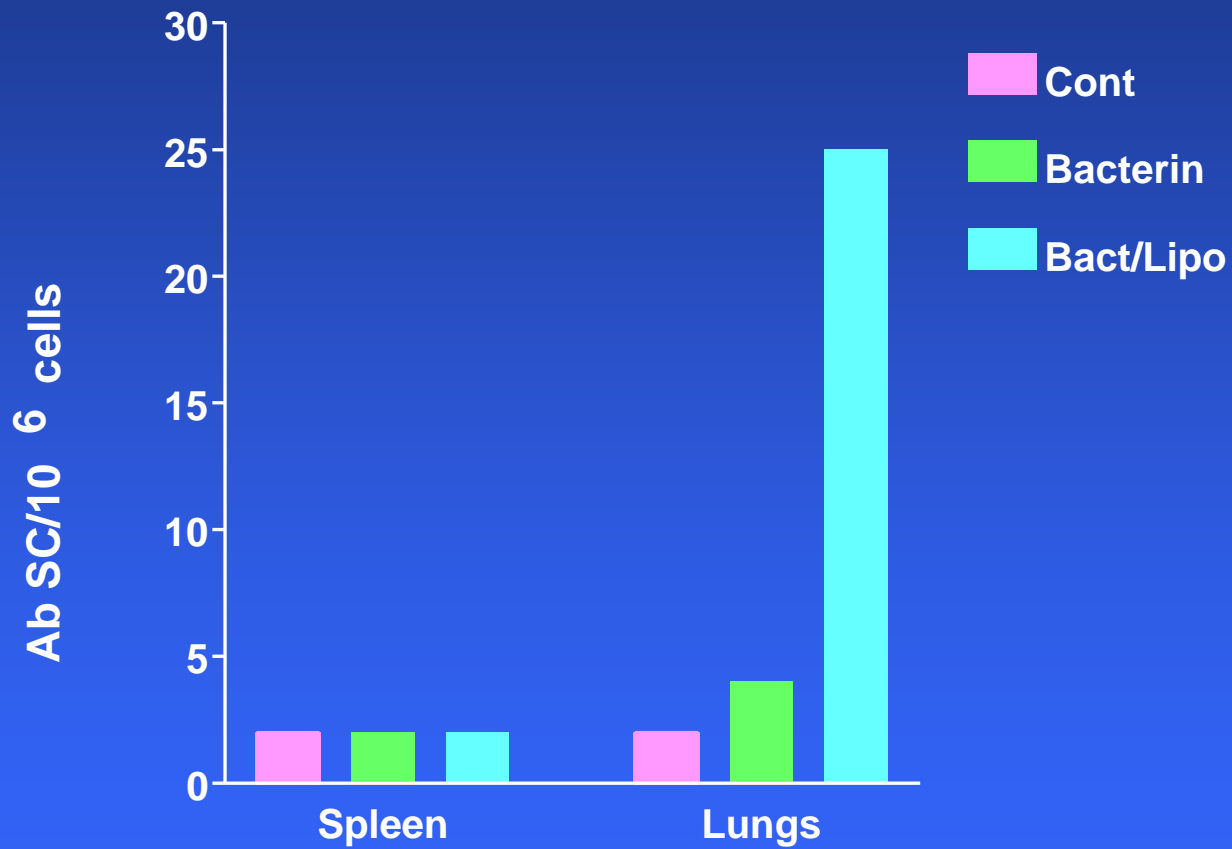
Bact 12.5 mg

Bact 12.5 mg/Liposomes

Antigen-specific IgA in Lung Washes



IgA ELISPOT



Experimental Approach

Objective: To assess the ability of LBDS to induce immune responses to OmlA antigen following intranasal administration



x 2 Intranasally



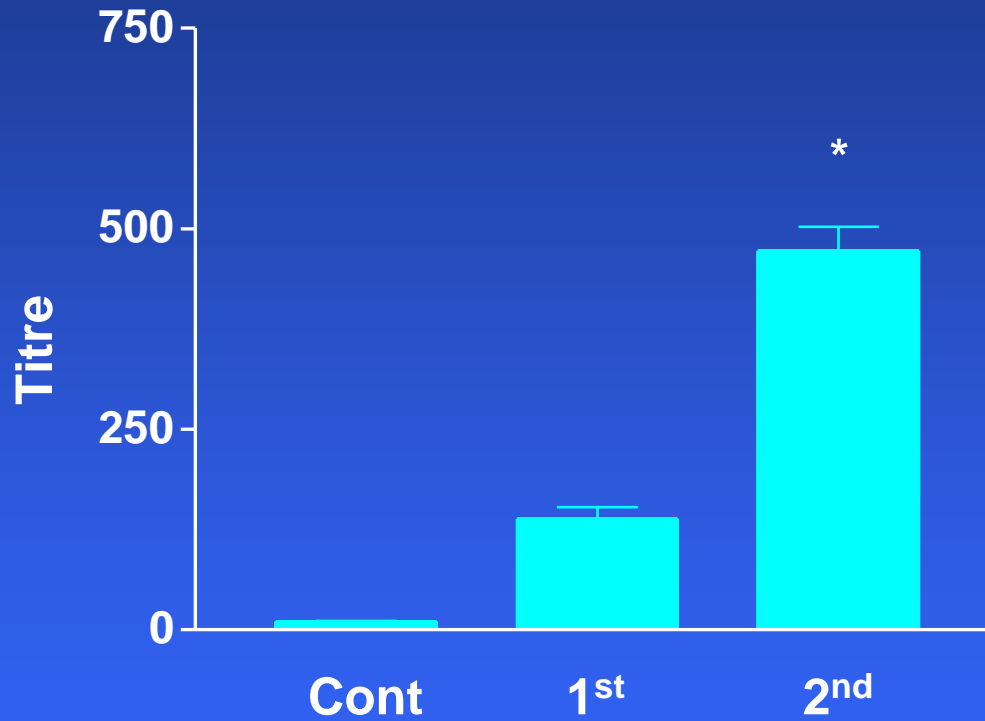
- Serum IgG

ANTIGEN

Actinobacillus pleuropneumoniae

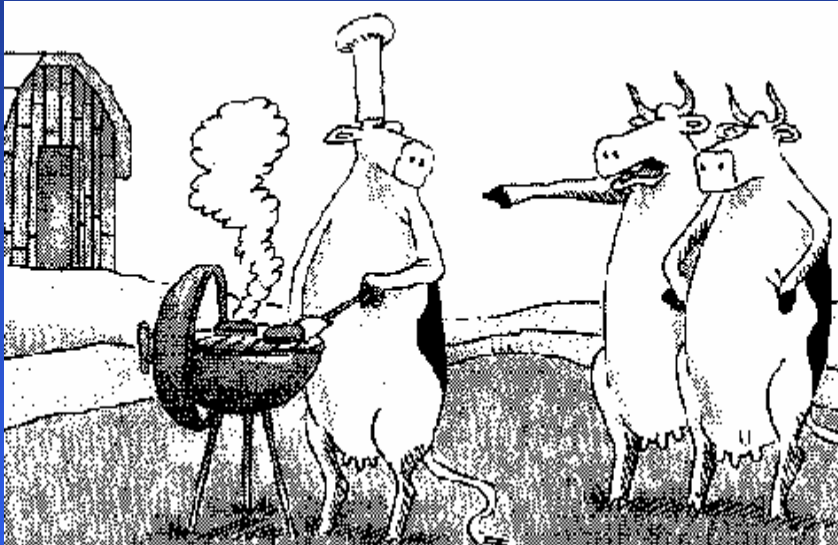
OmlA antigen

Anti-Oml-A IgG in Serum of Pigs Immunized Intranasally



Experimental Approach

Objective: To assess the ability of LBDS to induce immune responses to BHV-1 gD following intranasal administration



x 2 intranasally

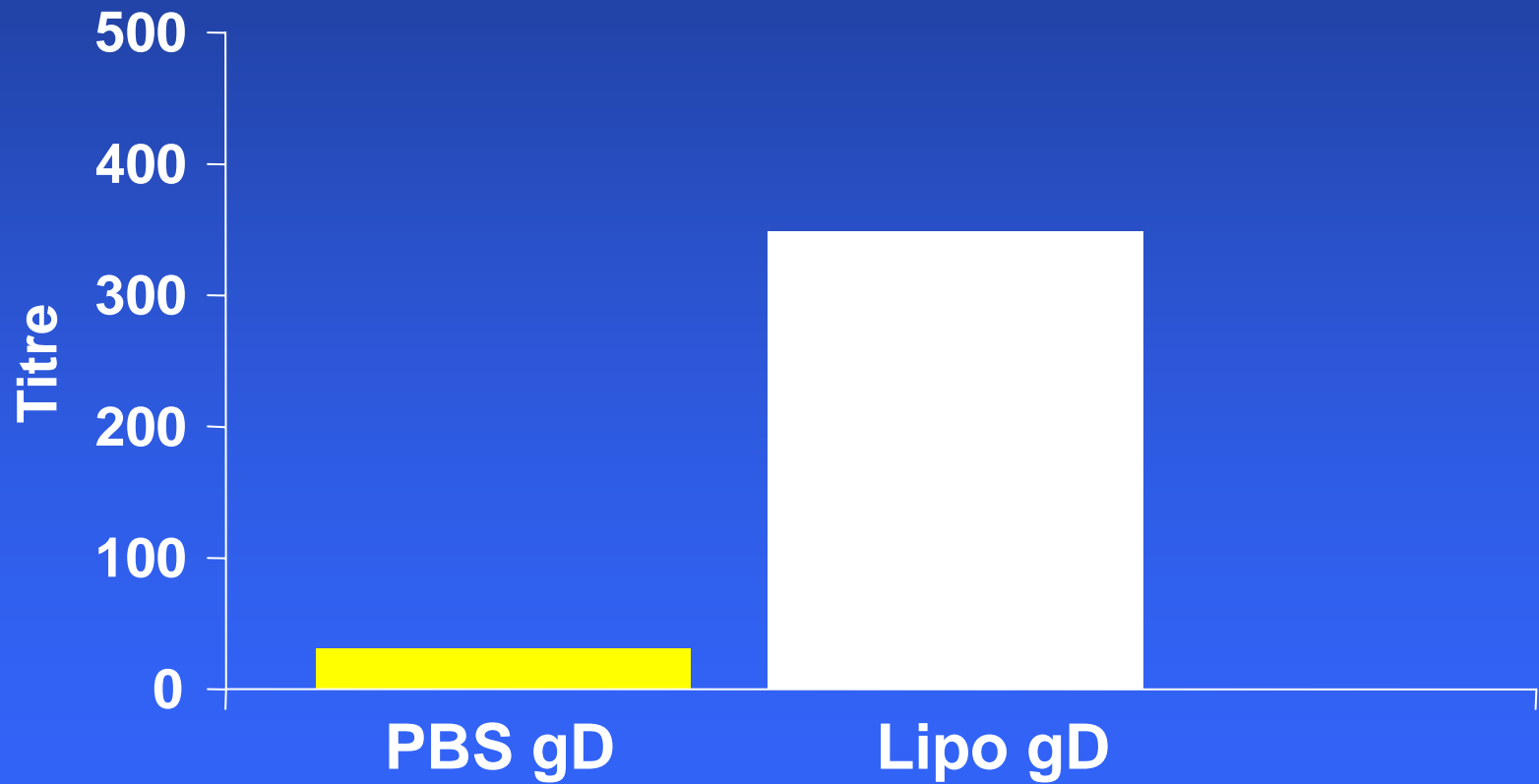


- Serum IgA
- Mucosal IgA
- BHV-1 Neut. Titres

ANTIGEN

Bovine Herpesvirus Type 1 (BHV-1)
glycoprotein D (gD)

Intranasal Immunization



APPROACHES TO LIVE-VECTORED VACCINE PRODUCTION

- Create gene deletions to attenuate
- Insert foreign genes into vector (chimera)

POTENTIAL VECTORS FOR LIVE RECOMBINANT VACCINES

VIRUSES

Poxviruses

- Vaccinia
- Fowlpox
- Canary pox

Adenovirus

Herpesvirus

Picornavirus

Flavivirus

BACTERIA

E. Coli

BCG

- bacille calmette-guerin

Salmonella

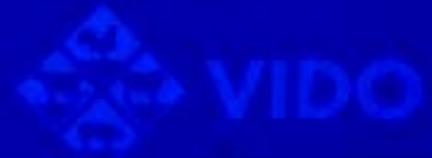
Lactobacilli

Gene Deleted Vaccine

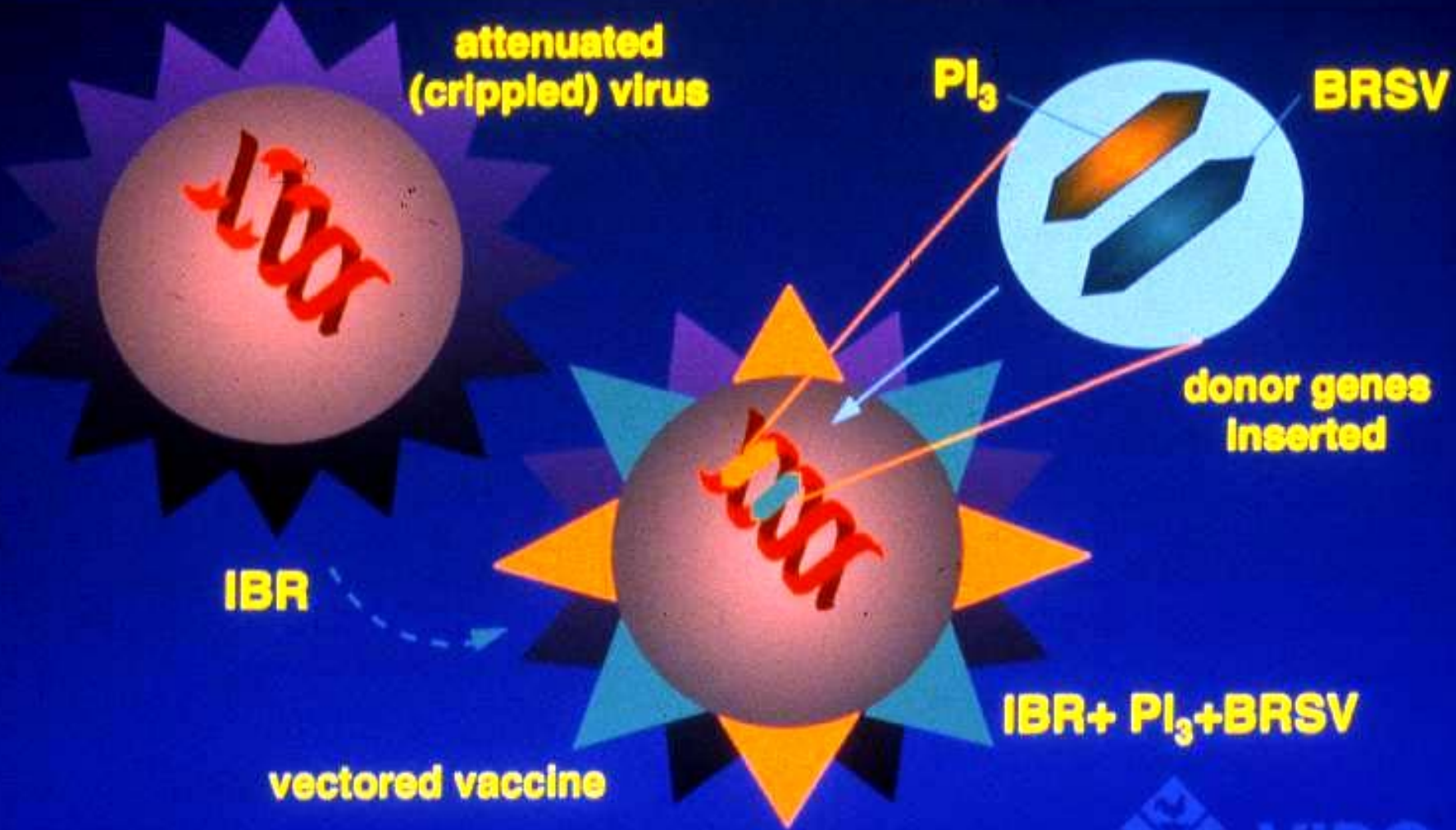
virus



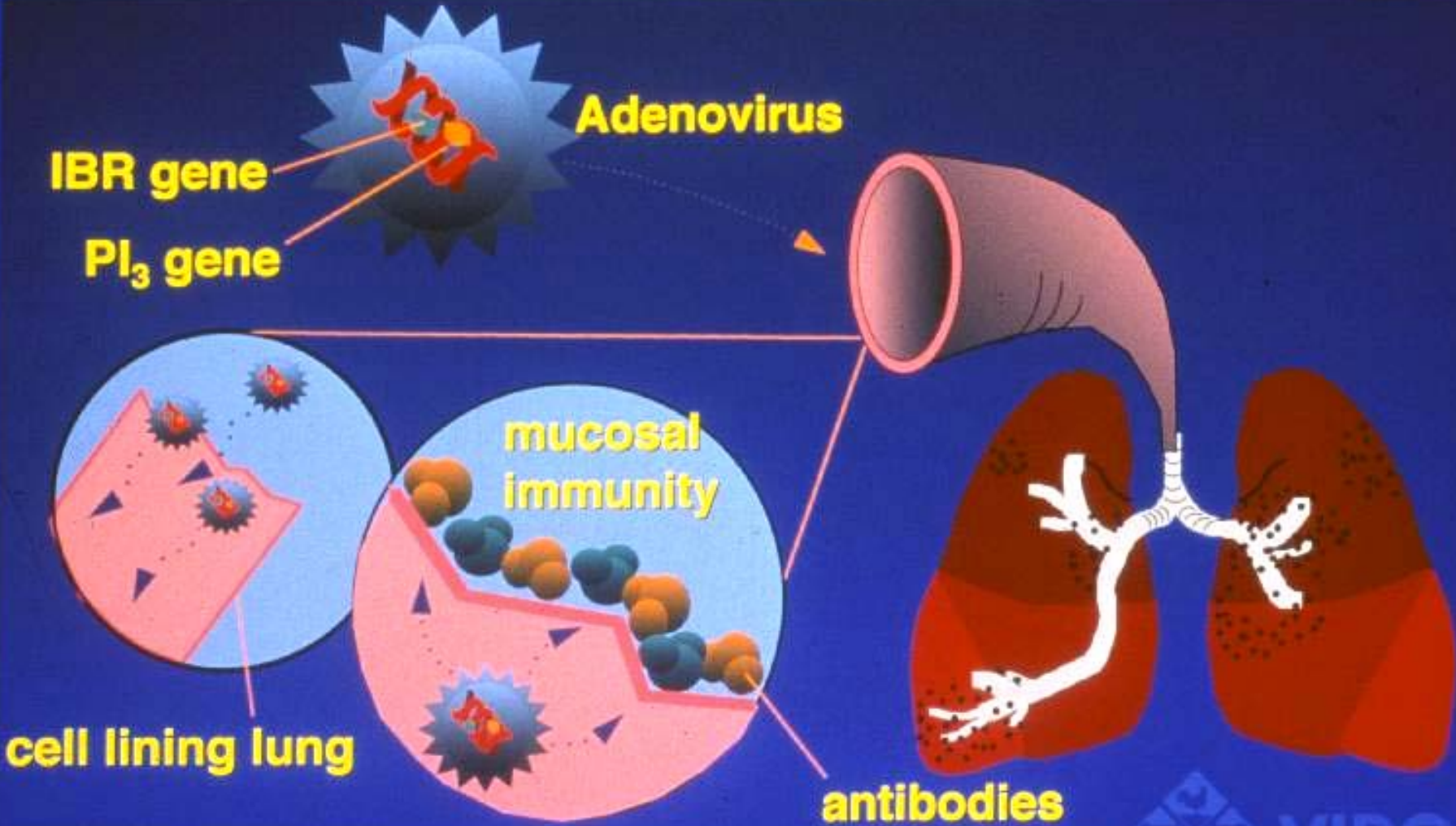
**attenuated
(crippled)
virus**



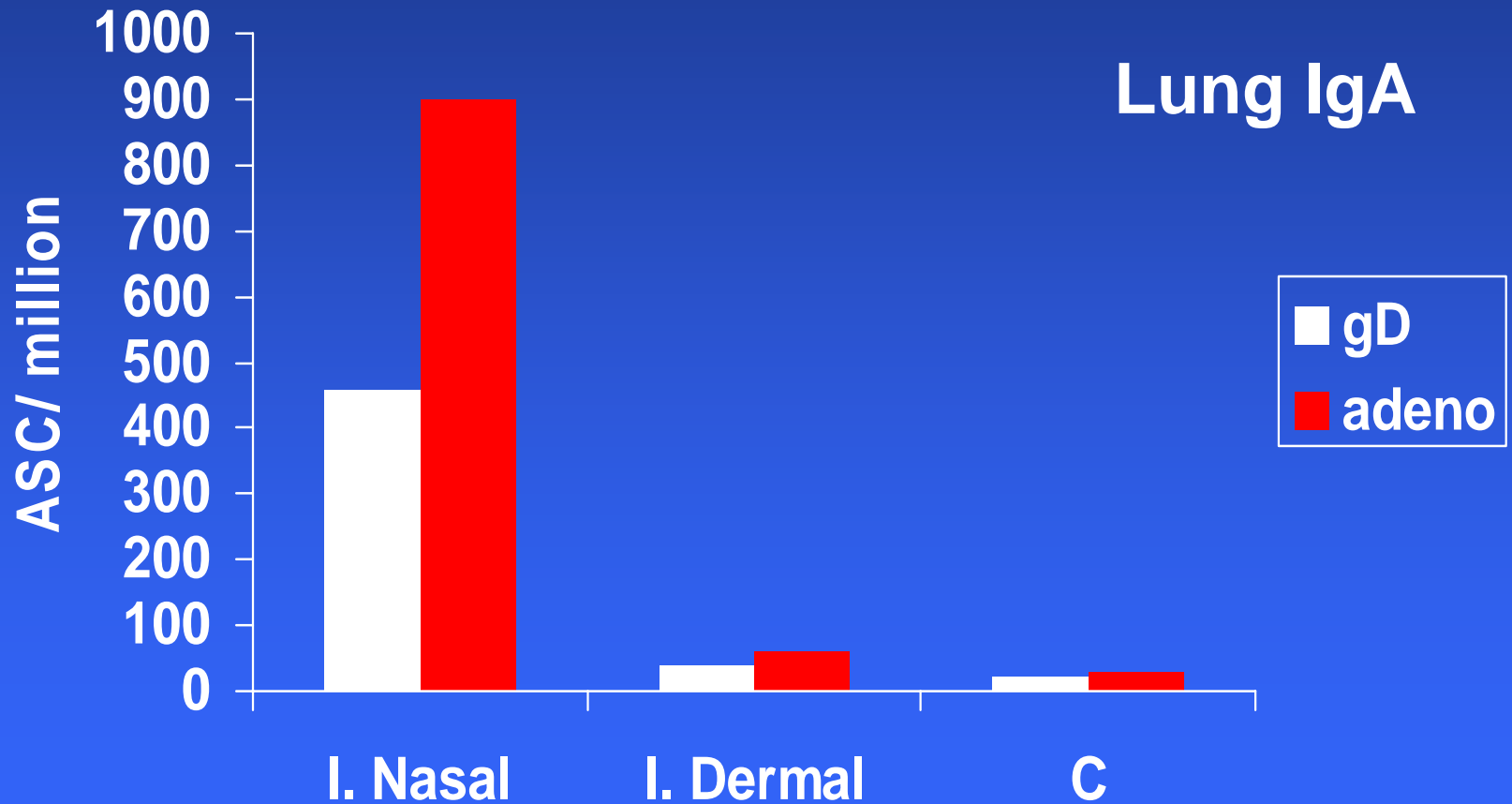
Vectored Vaccine



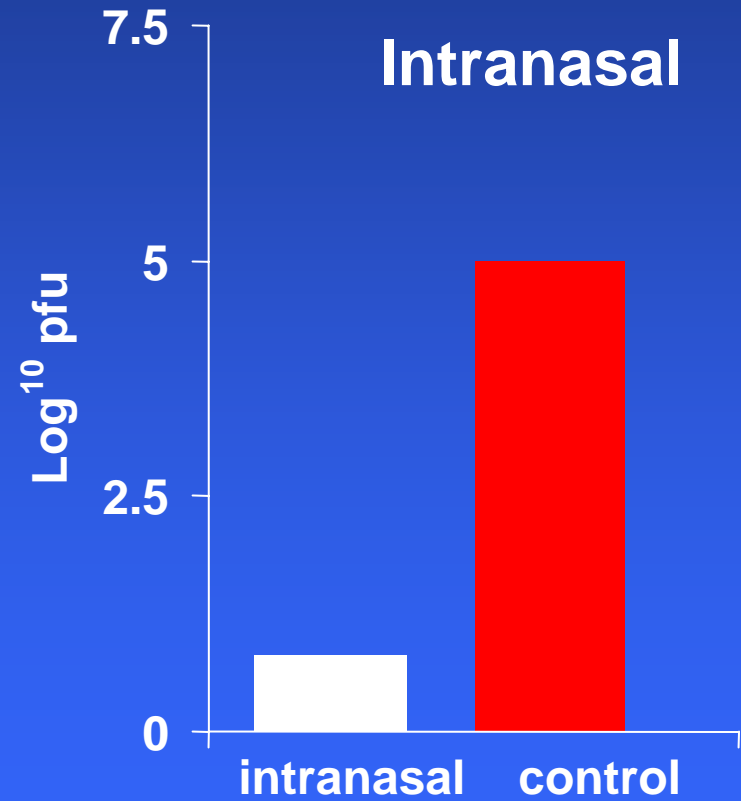
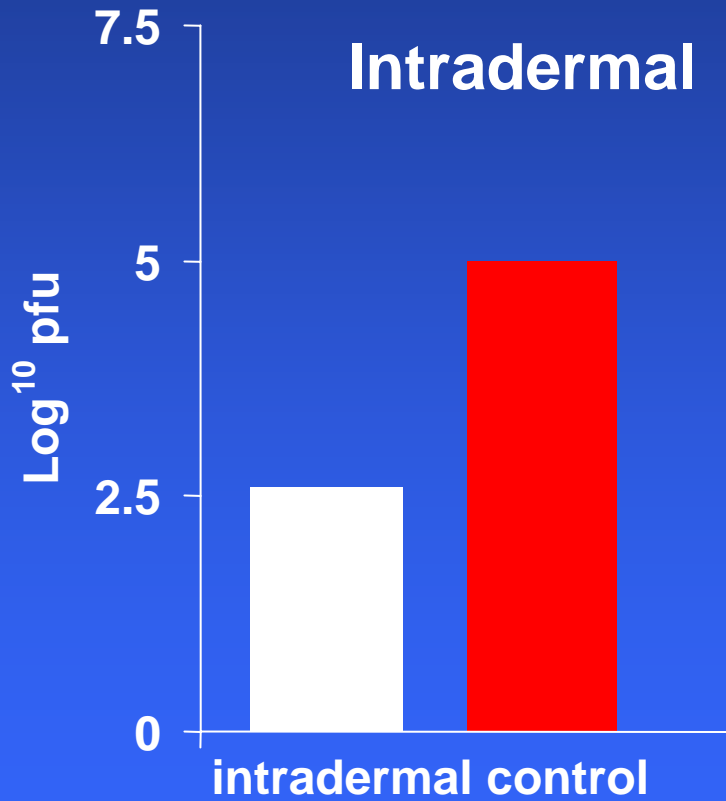
Mucosal Immunity



Antibody Secreting Cell Frequency in Lung-parenchyma Lymphocytes Measured by ELISPOT: Local Production of Mucosal IgA



RECOVERY OF BHV-1 FROM LUNG TISSUE



Summary

- **New technologies make the identification of vaccine antigens relatively straightforward and rapid.**
- **Technologies for the formulation and delivery of vaccines to stimulate mucosal immunity are a limiting step in our ability to make more efficacious vaccines.**
- **Regulatory and animal models are challenges to developing vaccines for emerging diseases**