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The Innovators:
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Saving Lives Worldwide: The Animal-Human Health Connection

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Developing Vaccines Against Difficult Pathogens

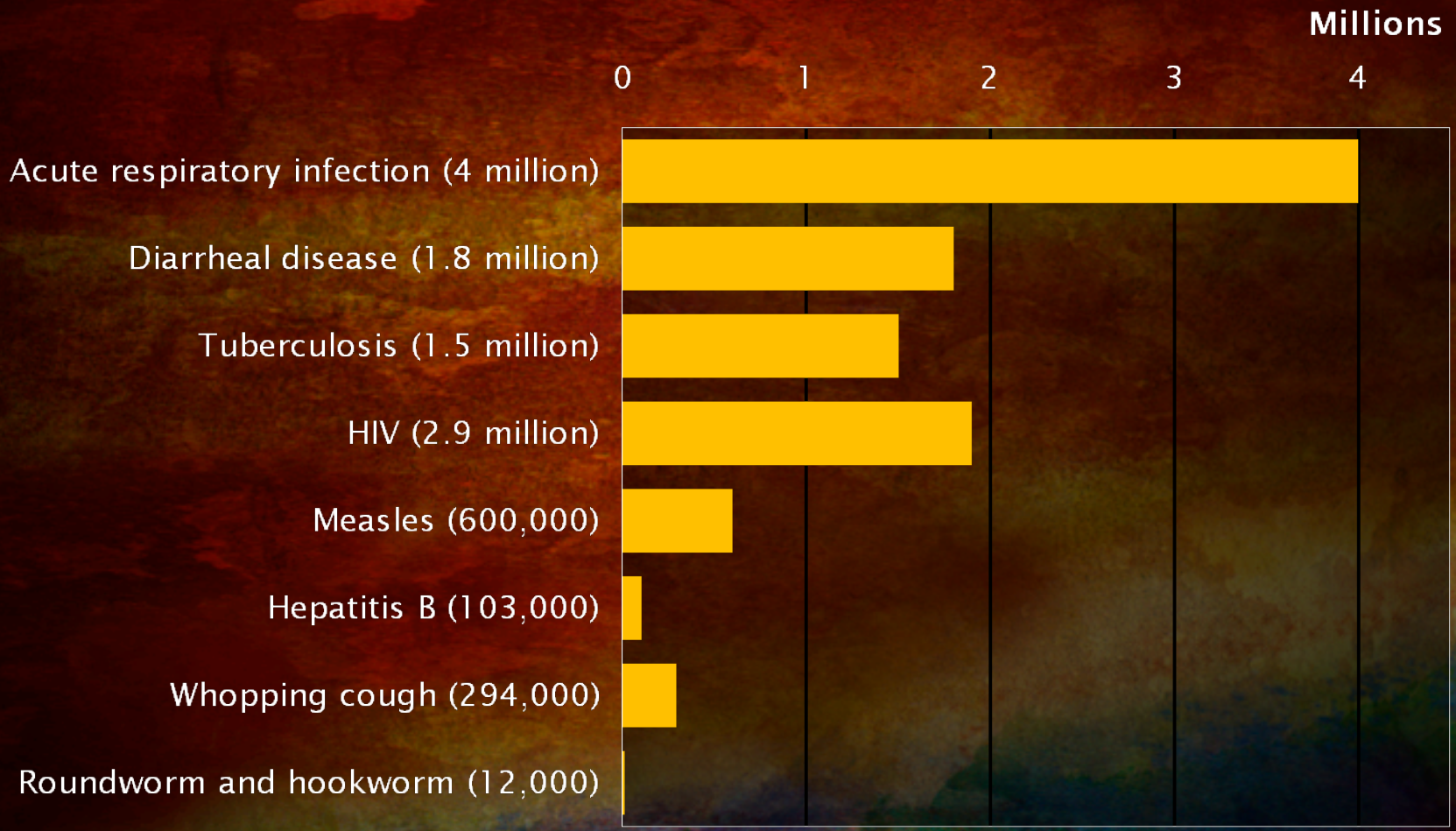
Outline

- Infectious Disease: human and animal impact
- What effective vaccines should achieve
- Examples of effective vaccines and how they work
  - Immunology 101
  - Example of vaccination against smallpox
- Why vaccines for many diseases have been difficult to develop
- Approaches to tackle the problems
- Examples of our research on vaccine development for anaplasmosis in cattle

Infectious Diseases

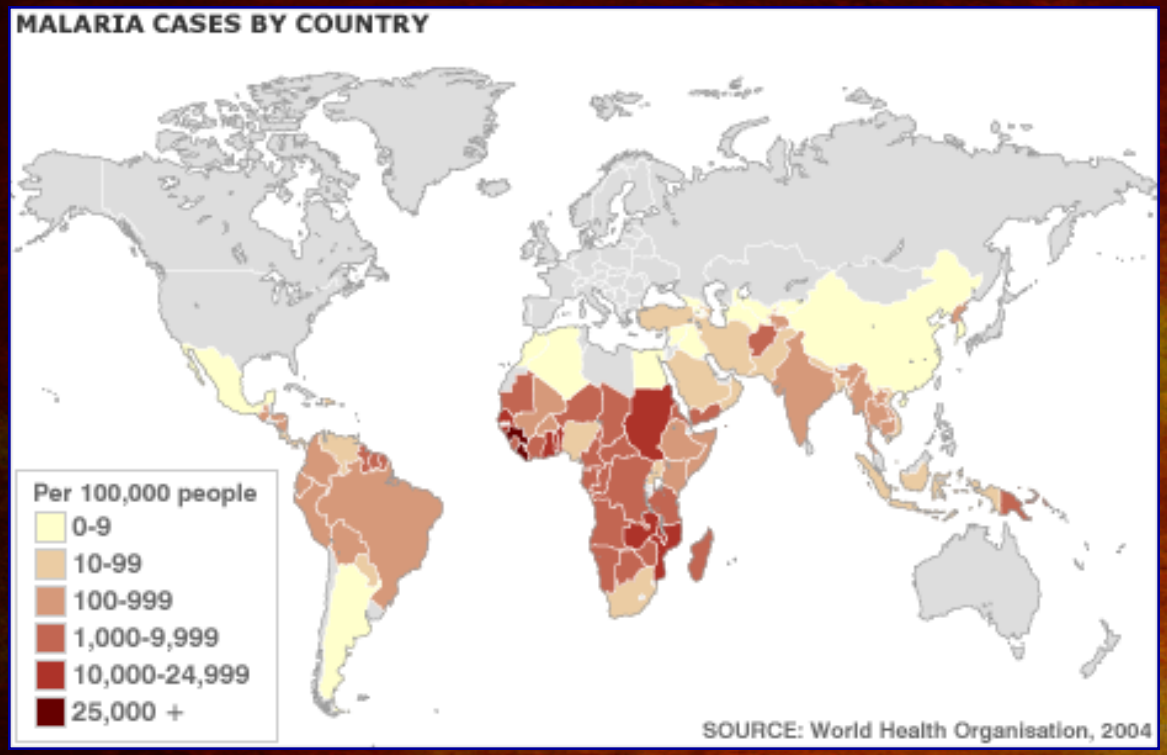
- Infection is the leading cause of death in humans worldwide
- Many diseases are preventable by improving sanitation and by vaccination
- In underdeveloped and developing countries, infectious diseases of humans and livestock take a heavy toll on human health and well being

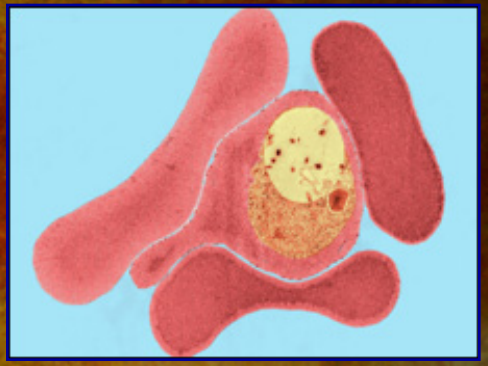
Annual Worldwide Deaths from Mucosal Infections (WHO 2002)



Immunobiology, Garland Science, 2008

Parasitic Diseases: Malaria





Estimated annual mortality:
~1.3 million people

The Animal-Human Health Connection: Agropastoralism

- East Africa smallholder dairy
  - 2-3 acres
  - 1-2 dairy cows
  - 2 goats
  - Grows all food for family and livestock

Cattle Tick-borne Diseases

- 80% of the world's cattle are at risk for one or more tick-borne diseases
- Global cost is estimated at $13.9-18.7 billion/year
- Diseases include babesiosis, anaplasmosis, heartwater, and East Coast fever

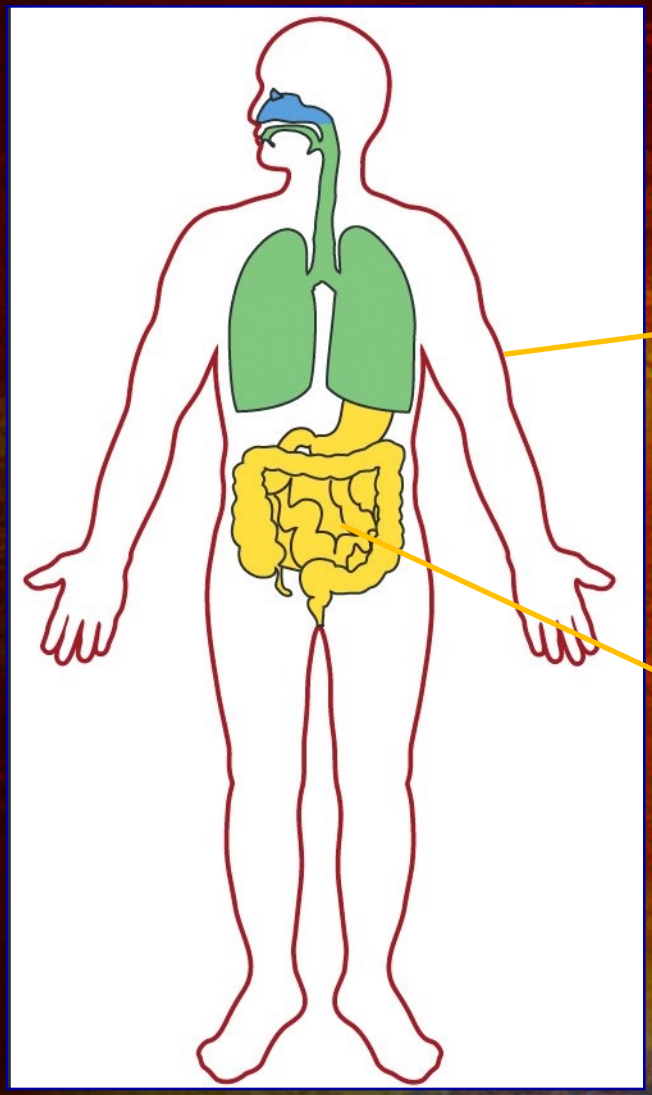
What Effective Vaccines Should Achieve

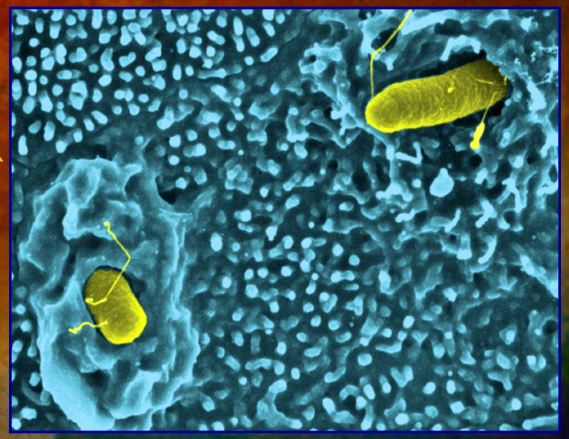
- Safety
- Induce protection in high percentage of vaccinated individuals
  - Provide herd immunity
  - Reduces transmission if large % are not infected
- Generate long-lived immunity (memory)
  - One or few immunizations
  - May mimic a naturally acquired immune response that clears the infection
- Cost-effectiveness
  - Affordable in underdeveloped countries
  - Able to be administered in rural areas

Microbes and Pathogens

- Different types of microbes
- Why do some cause disease but not others
- Innate immunity
- Breaching or evading the innate immune response
- Need adaptive immunity to fight and clear infection

Barriers to Infection by Pathogens





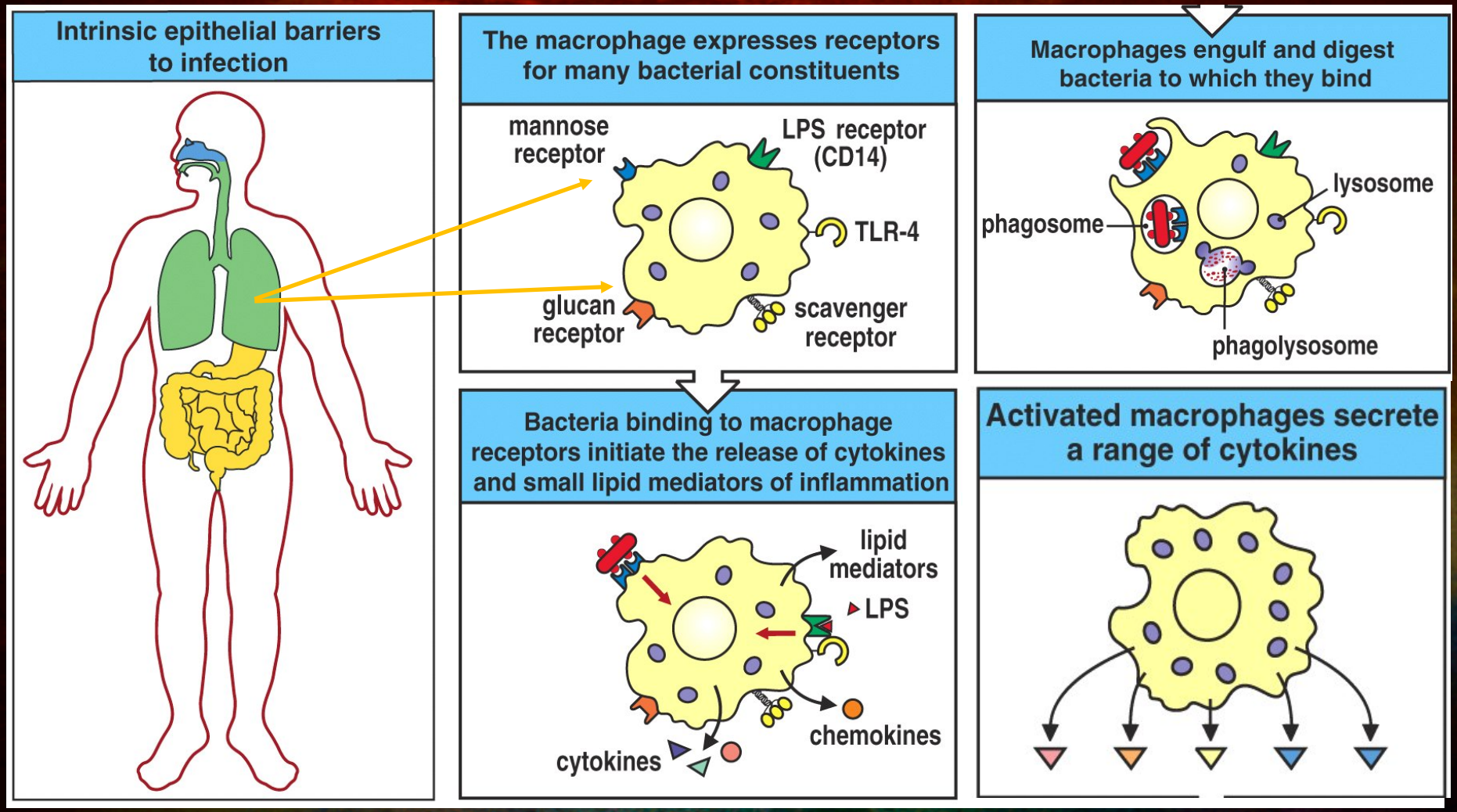
Salmonella typhi

Immunobiology, Garland Science, 2008

Pathogen-associated Molecular Patterns (PAMPs) and Adjuvants

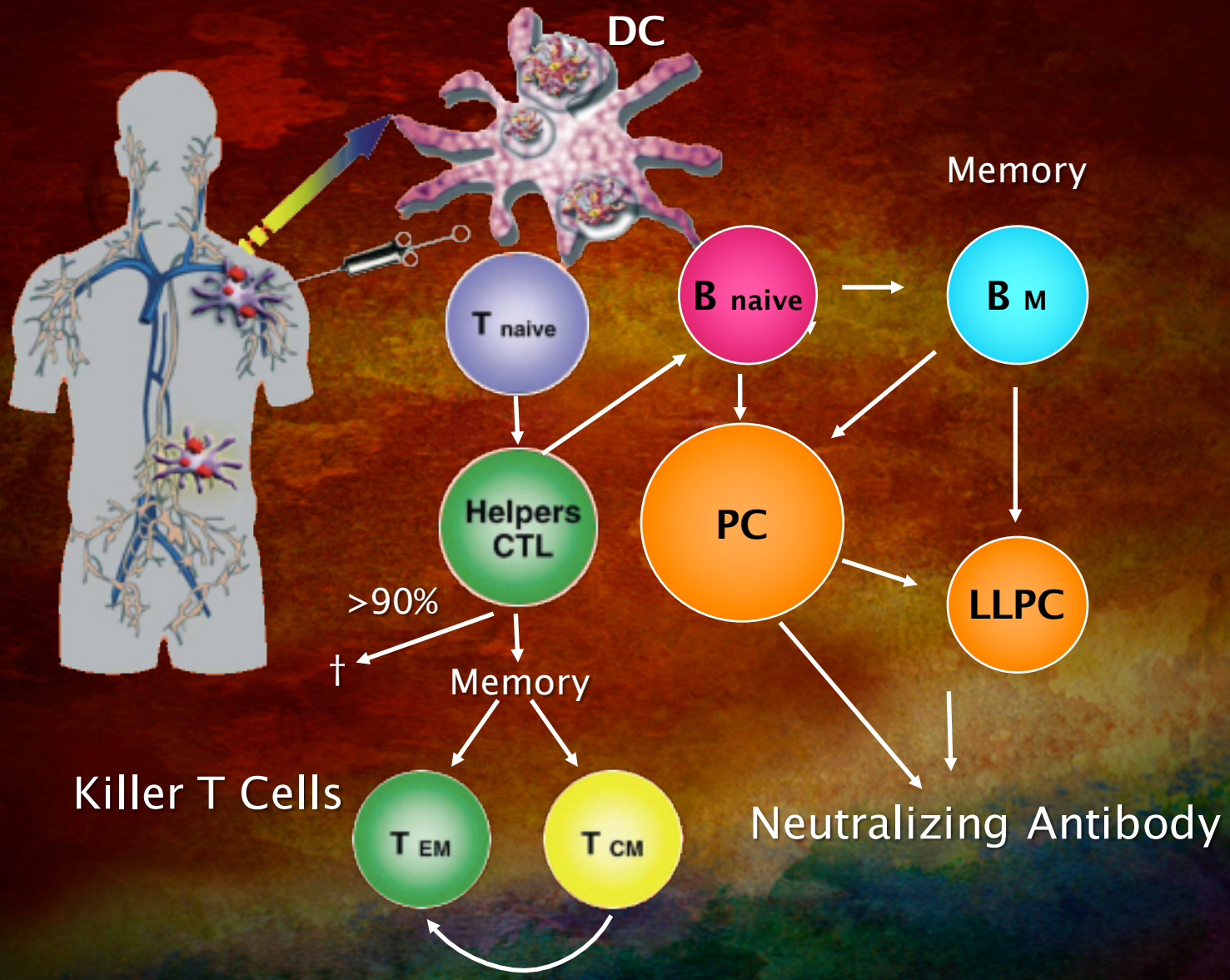
- Most pathogens activate the innate immune system through molecules they express
  - Surface lipopolysaccharides (LPS)
  - Bacterial cell wall components
  - Bacterial flagellin
  - Nucleic acids
- Live, attenuated pathogen vaccines have natural adjuvants
- Adjuvants were developed to use with killed vaccine antigens to mimic this ability to stimulate innate immunity

The First Line of Defense—Innate: Activation of Macrophages



Immunobiology, Garland Science, 2008

Adaptive Immunity and Memory



Modified from Esser et al., *Vaccine*, 2003

Examples of Diseases for which We Have Successful Vaccines

- Smallpox
- Measles, mumps, rubella (MMR)
- Chickenpox
- Influenza
- Polio

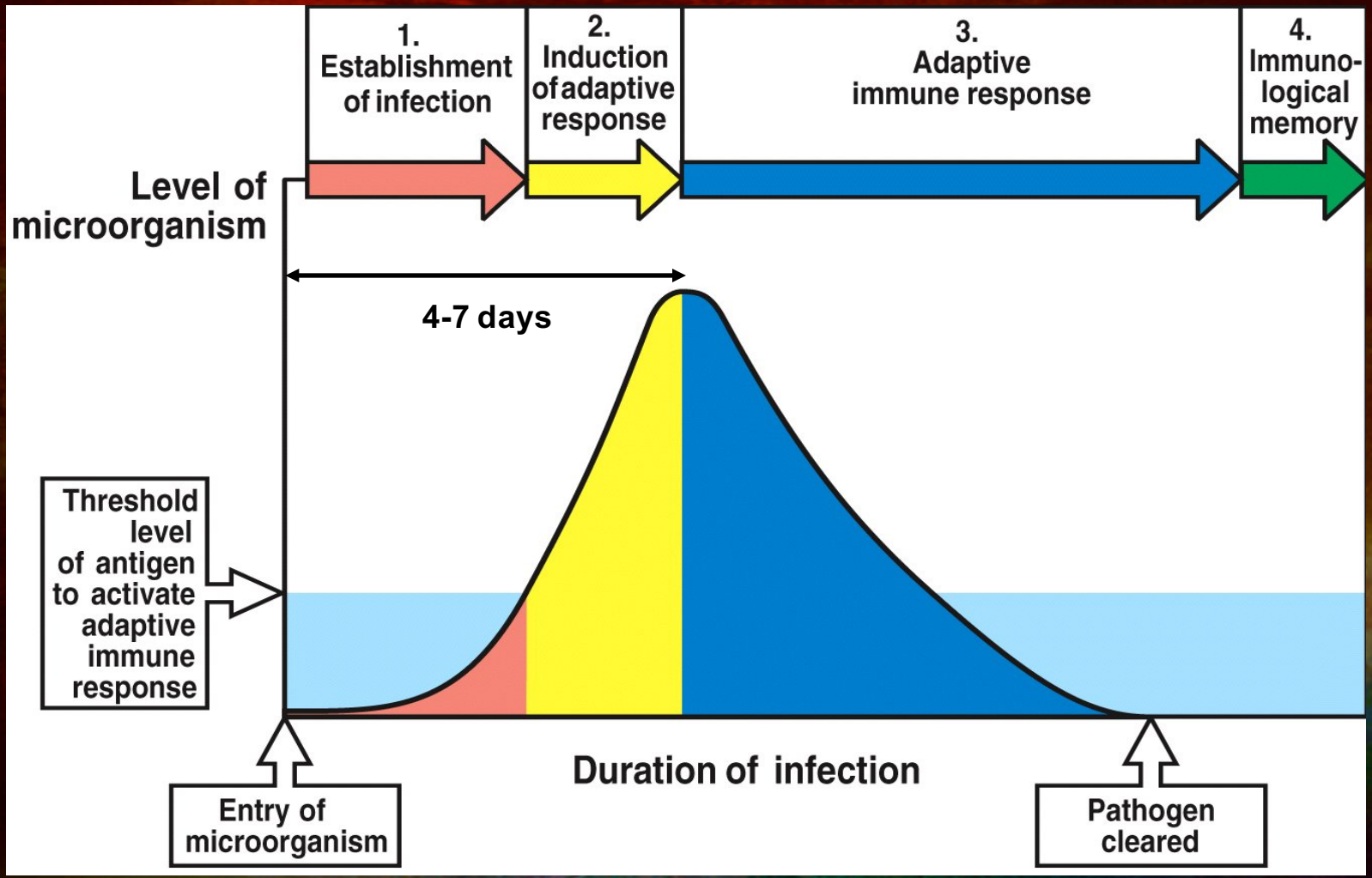
- Diphtheria, tetanus, pertussis (DTP)

- *Streptococcus pneumoniae* pneumonia
- *Haemophilus influenzae* meningitis

What Do These Diseases Have in Common?

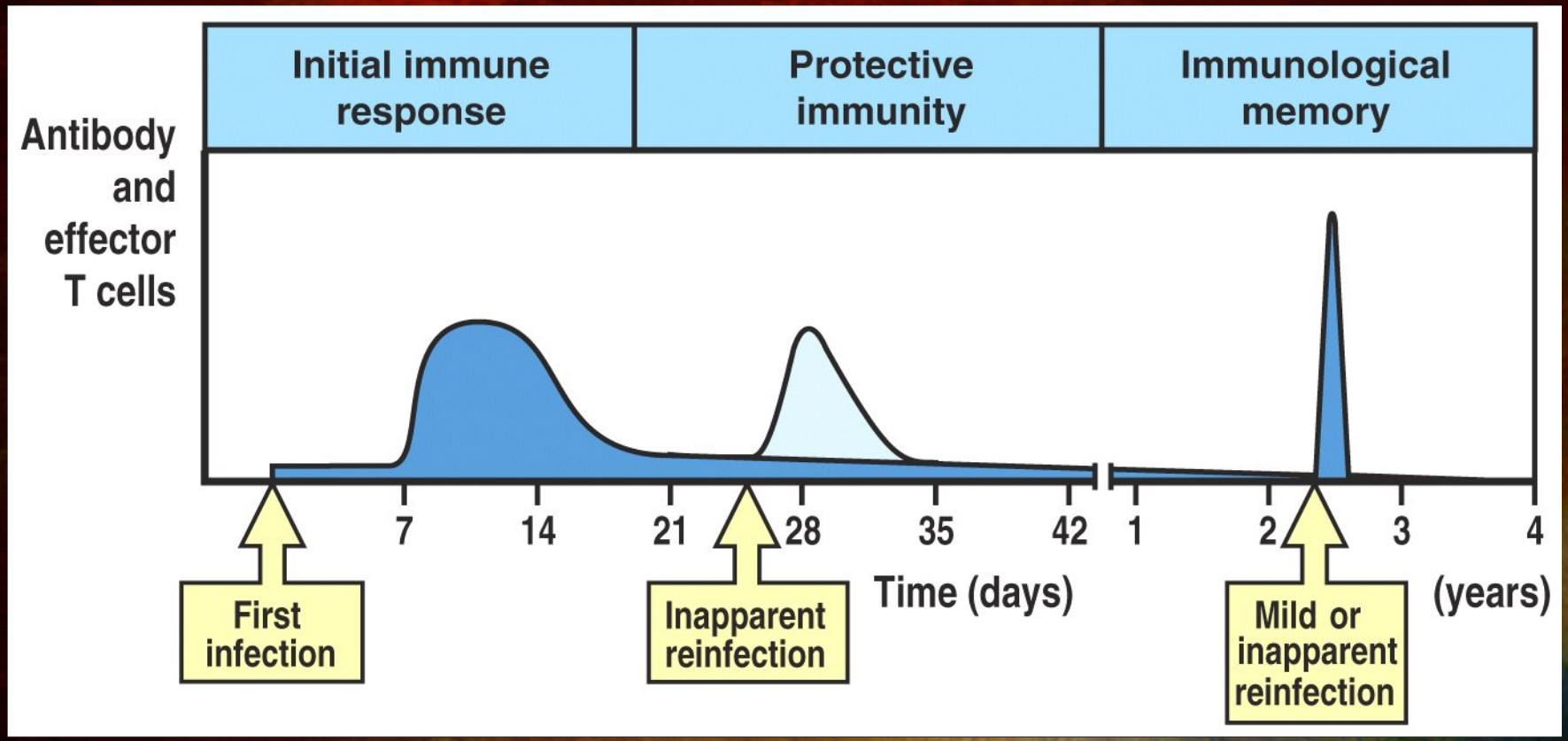
- Pathogen induces an innate immune response
- Acute infections can be cleared naturally by the host adaptive immune response
- Susceptible to neutralizing or antibody
- Viral infections can be cleared by killer T cells
- Immunization provides long-lasting immunity

Course of an Acute Infection Cleared by the Immune Response



Immunobiology, Garland Science, 2008

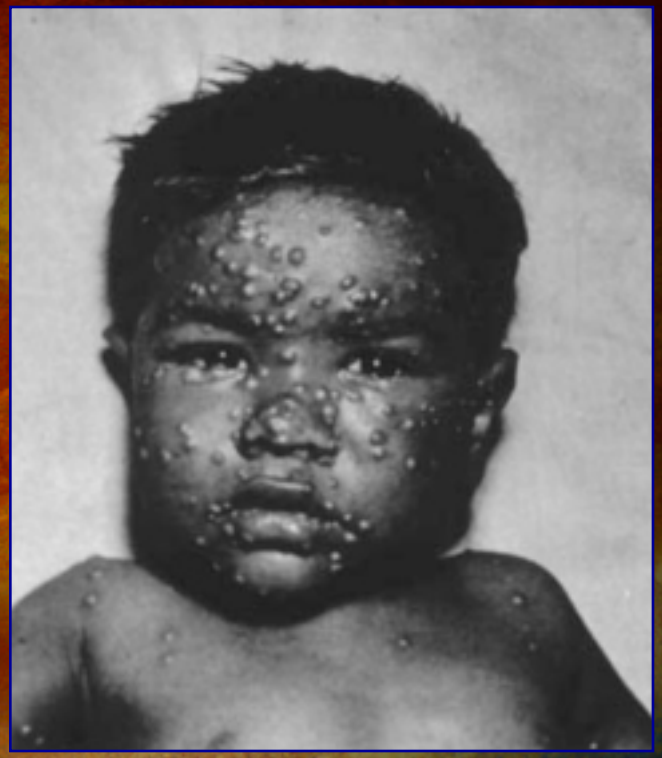
Long-term Protective Immunity = Preformed Immune Reactants + Memory



Immunobiology, Garland Science, 2008

History of Smallpox

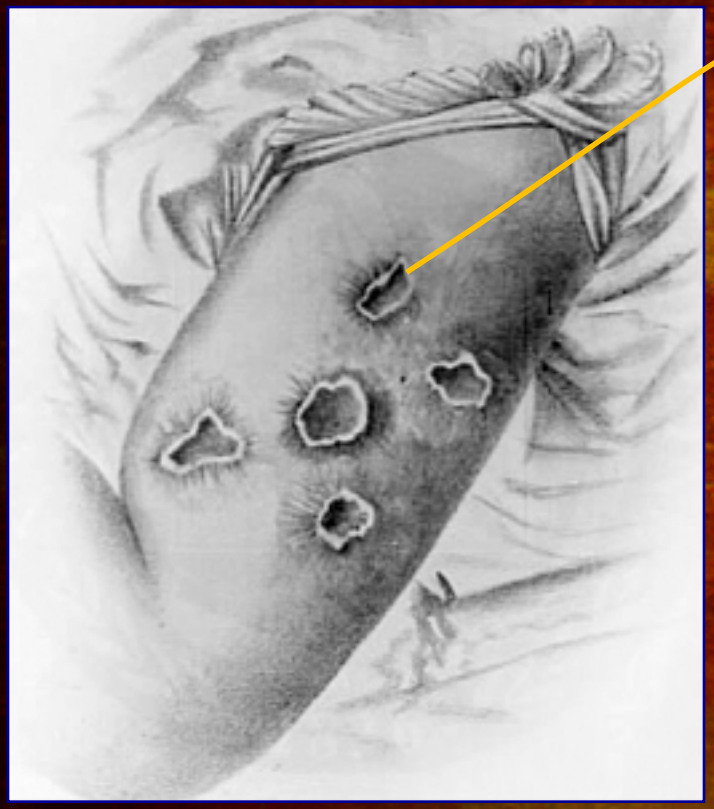
- Viral infection — variola virus
- Present in Africa, Asia, and Europe since at least 400 BCE
- Disease spread along trade routes
- Introduced to the Americas in the 1500s
- Highly contagious viral disease, spread by respiratory route, ~30% mortality rate
- Eradicated by 1980



Immunization Against Smallpox: Variolation

- Deliberate inoculation of dried smallpox scabs into the nose or skin in the 1700s
- Caused a mild form of disease
- Lifelong immunity
- 1-2% mortality rates
- Immunized individuals could still spread the virus

Immunization Against Smallpox Using Cowpox Virus: Vaccination



Arm of Sarah Nelmes, Dairy Maid, 1796

Inoculate James Phipps, 8-yr-old boy

↓

Infect with smallpox

↓

Protected

Edward Jenner, English Physician

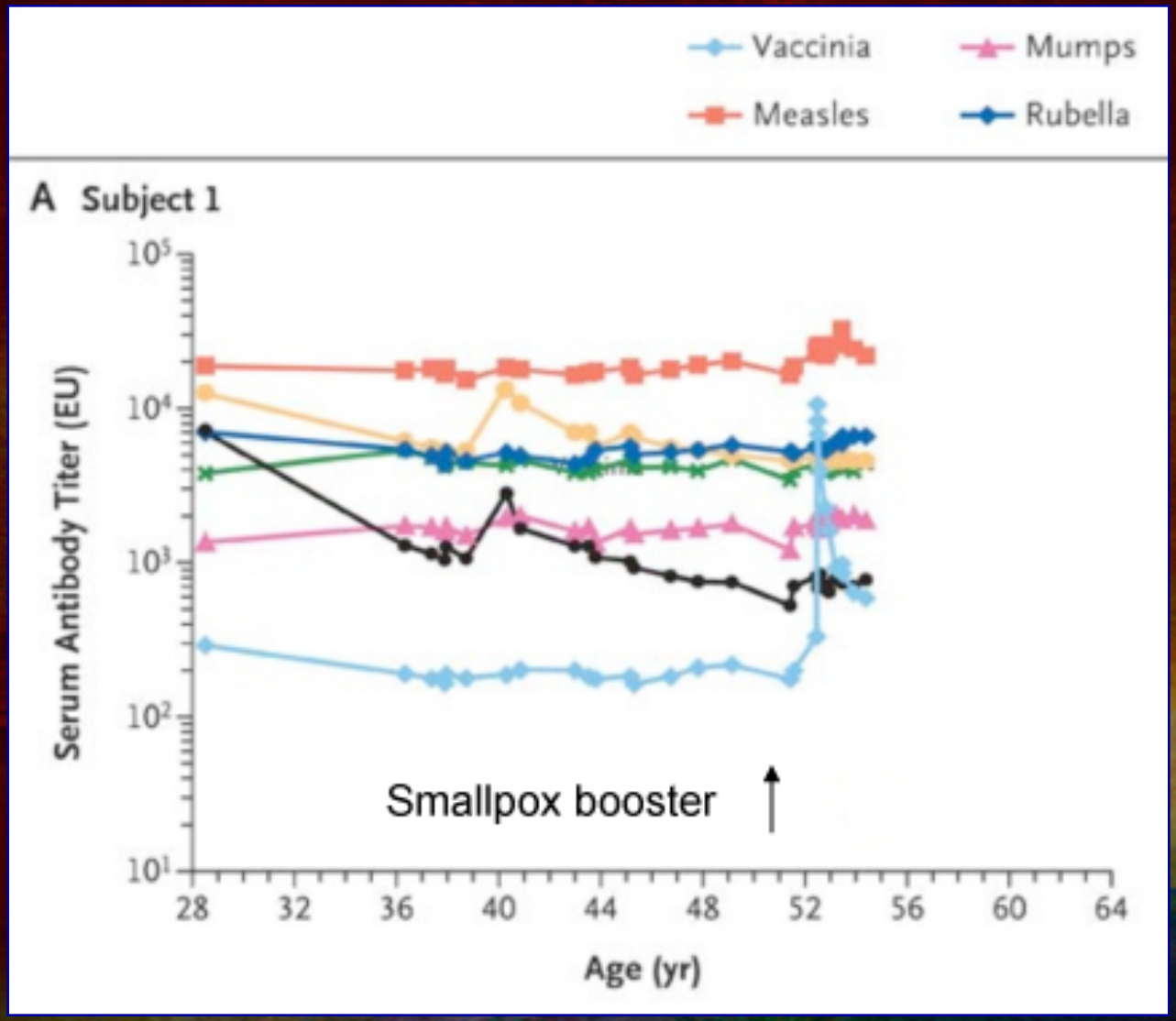
Immunization Against Smallpox: Vaccinia Virus

- Poxvirus related to cowpox and smallpox
- Attenuated virus—causes mild, unapparent infection in normal individuals
- Live vaccine protects against smallpox

Why Is Vaccinia Virus a Great Vaccine?

- Live, attenuated virus
- Natural adjuvant properties
  - Potent activator of the innate immune system to secrete anti-viral proteins (interferons)
  - Activates through TLR2 to induce a strong inflammatory response
- Very broad response to viral proteins
- Long duration of anti-viral immunity
  - Antibody response is stable up to 75 years
  - T cell responses still detectable as well

Long-lived Serum Antibody Levels



Amanna et al., *New England Journal of Medicine*, 2007

Summary — Pathogens for Which Protective Vaccines Exist

- Pathogens can be naturally cleared by the immune response – do not persist
- Tend to have adjuvant properties
- Induce neutralizing antibody
- Induce long-lasting immunity
  - Memory T cells
  - Memory B cells
  - Long-lived plasma cells

Diseases for Which Vaccines Are Needed

- Tuberculosis
- Trypanosomiasis
- Malaria
- Rickettsial diseases
- Diarrheal disease
- Respiratory infections
- HIV/AIDS
- Measles

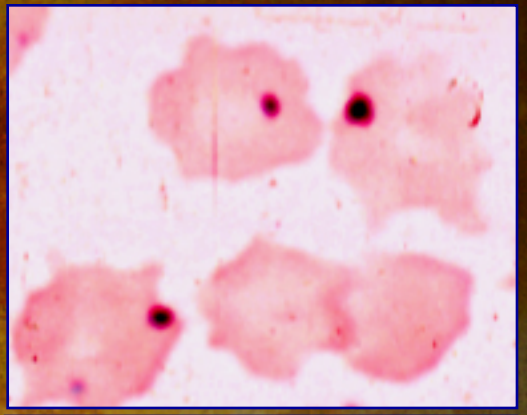
- Tuberculosis
- Trypanosomiasis
- Babesiosis
- Rickettsial diseases
- Diarrheal disease
- Respiratory infections
- Foot and mouth disease
- Rinderpest

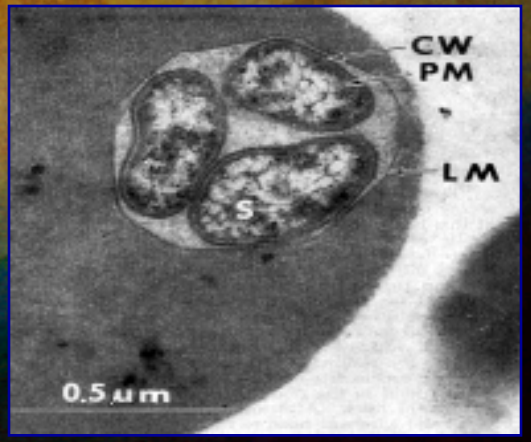
Some Reasons Vaccines are so Hard to Develop

- Intracellular
- Deactivate the innate defense mechanisms
- Rapid onset of systemic infection before adaptive immunity can work
- Antigenic variation in surface proteins
- Cause persistent infection
- High antigen loads: deletion of effector cells

Anaplasma marginale

- Most prevalent tick-borne pathogen in cattle worldwide
- Obligate intracellular bacterium
- Acute febrile illness with severe anemia; up to 50% mortality
- Antigenic variation in MSPs
- Lifelong persistent infection
- Outer membrane immunization can prevent disease and infection
- Immunodominant surface MSPs are not protective



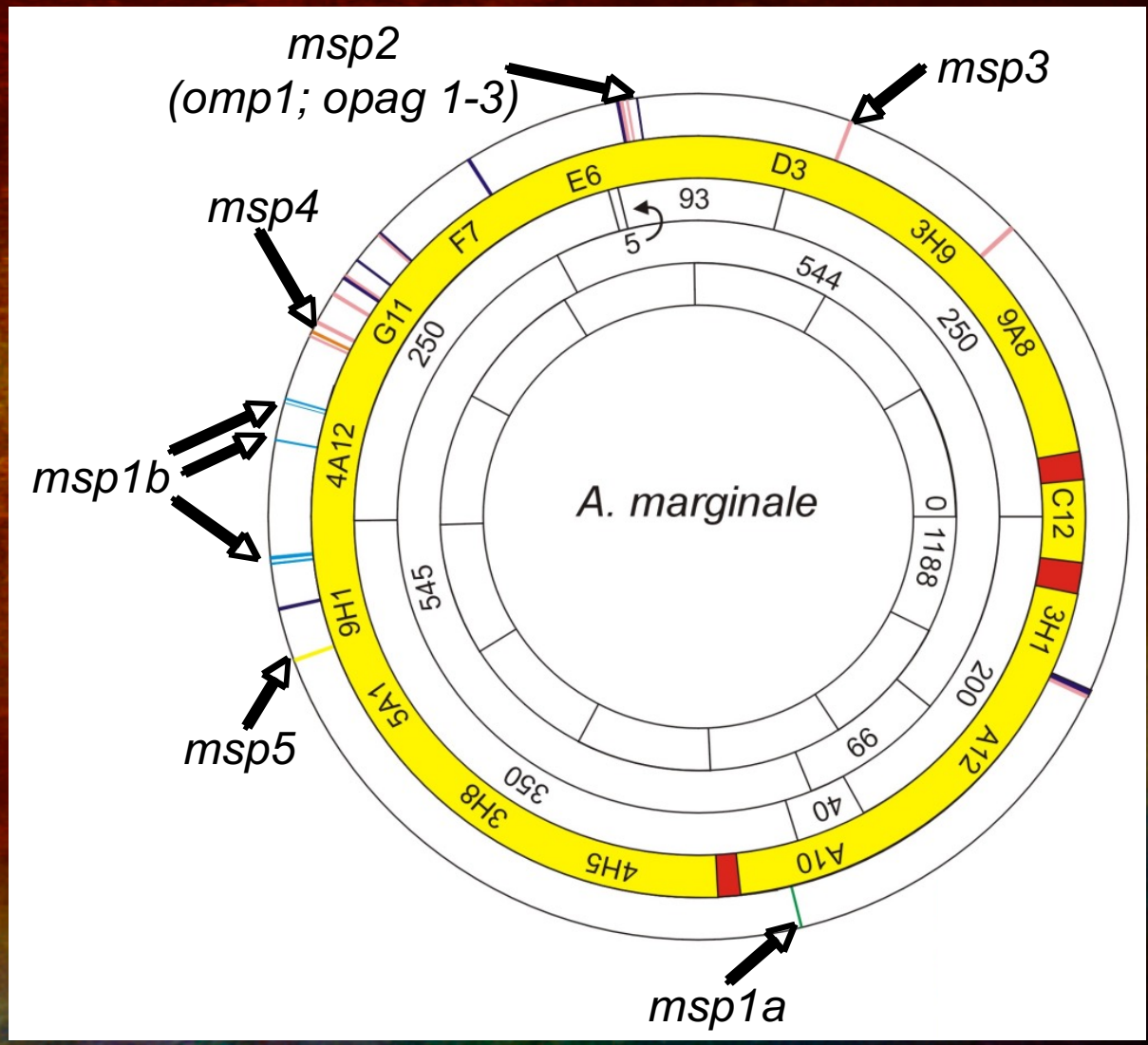


Selection of Vaccine Antigens Using Immune Effectors from Outer Membrane Vaccinates

- Proteomic: identify proteins that stimulate immune effectors and map to the genome to identify the encoding gene
- Genomic: identify genes predicted to encode outer membrane proteins—test expressed proteins for stimulation of immune effectors from protectively immunized cattle

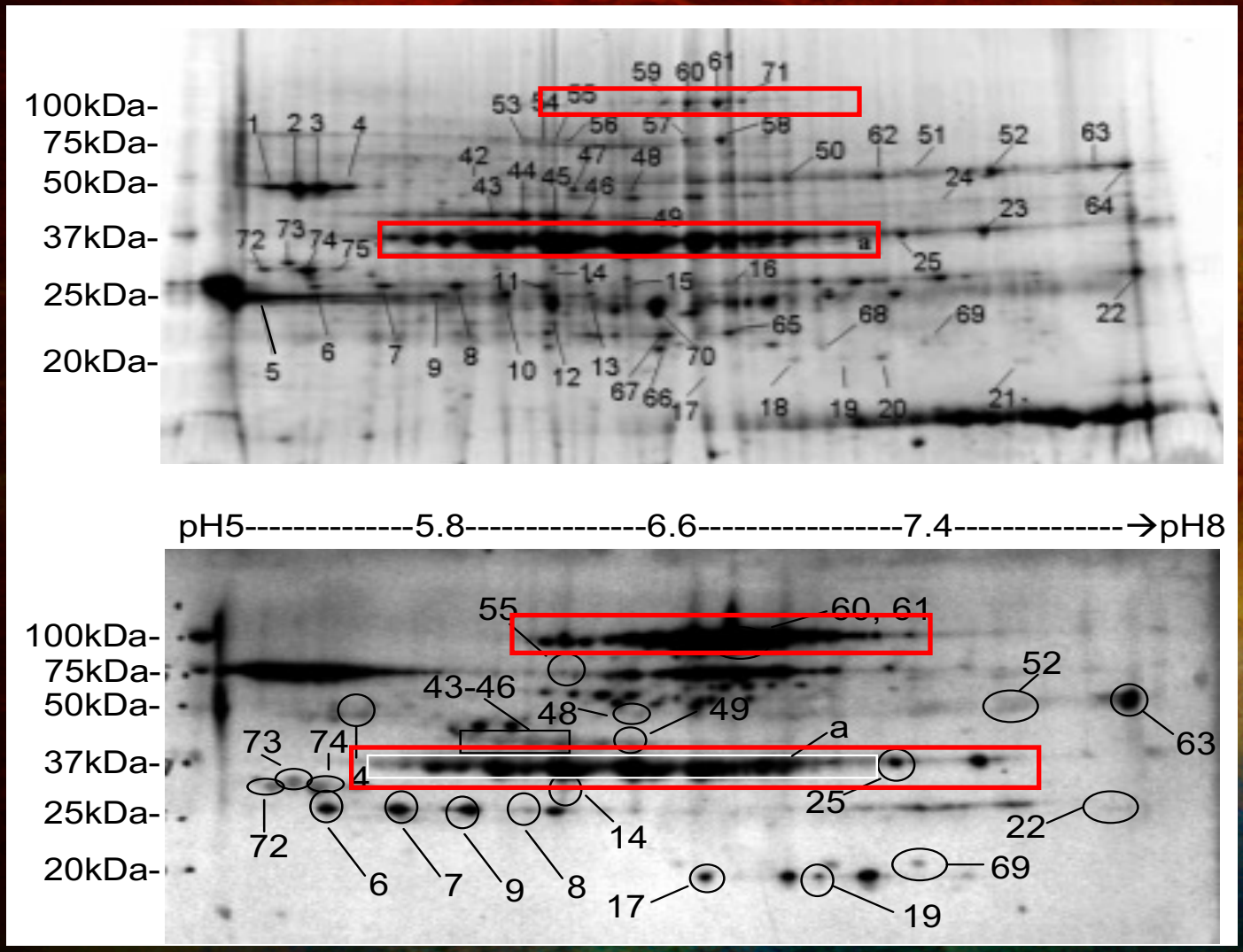
Anaplasma marginale Genome

- 62 predicted OMPs
- 12 OMPs characterized
- None gave adequate protection



Brayton et al., *PNAS, 2005*

Hypothesize Subdominant Antigens Are Better Vaccine Candidates



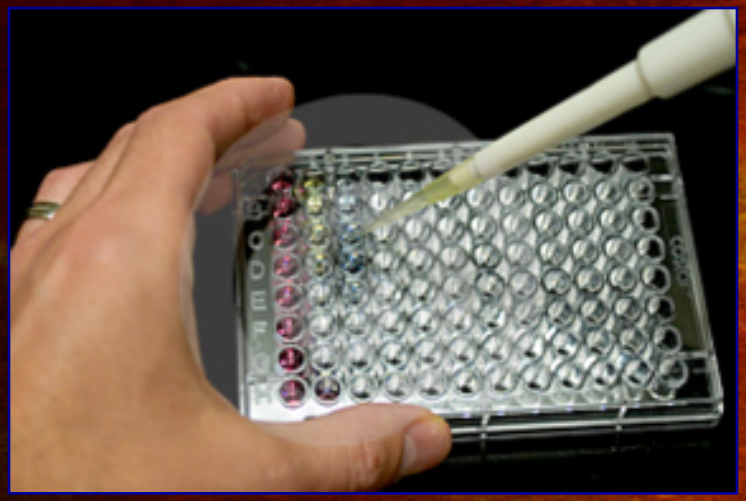
2-D Gel

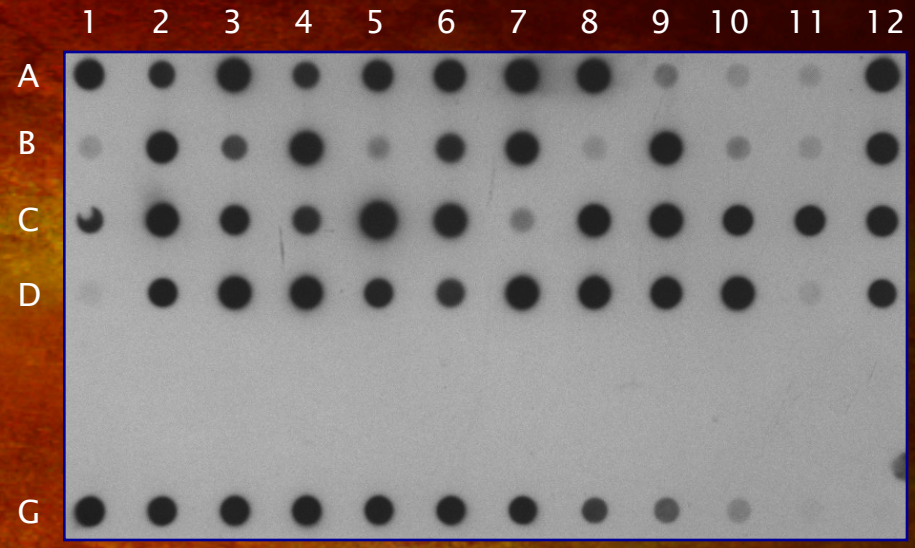
Immunoblot

>20 new Ags Stimulate Antibody

Lopez et al., *Infect. Immun.*, 2005

High-throughput Gene Expression by IVTT

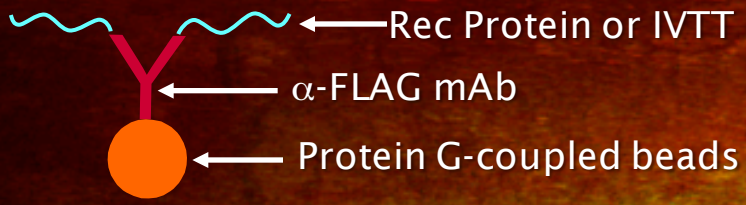




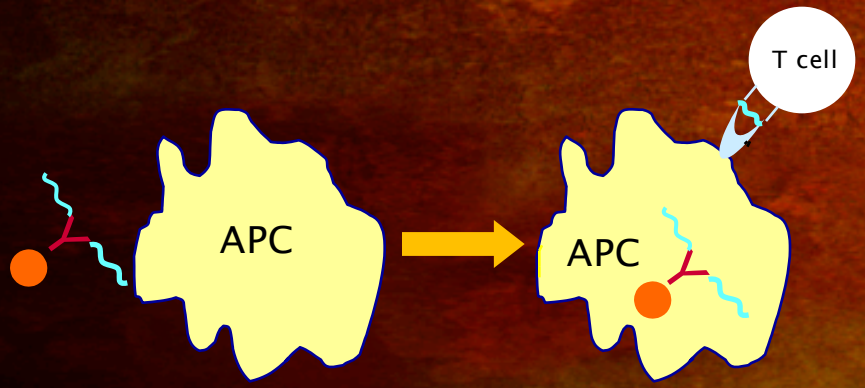
Probed with AP anti-FLAG antibody

1. Clone genes of interest
2. Add FLAG epitope to C-terminus of protein
3. Express in 96-well plates by IVTT
4. Measure protein expression with anti-FLAG antibody

Stimulation of Immune CD4 T Cells with IVTT Proteins

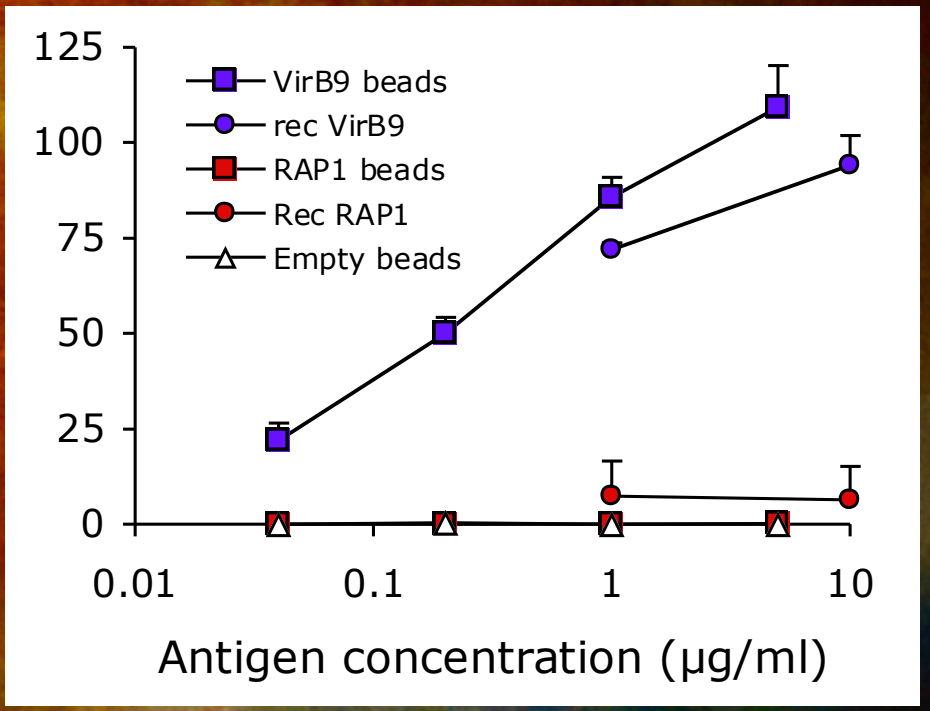


Mix, wash, add to APC + T cells



Volume of beads adjusted to [Protein G]

Proliferation assay



Lopez et al., *J. Immunol. Meth.*, 2008

High-throughput Screening of Antigens Expressed by IVTT

- ~60 proteins tested
  - Selected by genomic annotation as membrane proteins
- Verified 6 known antigens
- Identified 20 new proteins stimulated significant T cell proliferation from OM vaccinates
- Can be accomplished in weeks

Lopez et al., *J. Immunol. Meth.*, 2008

Conclusions

- The proteomic approach using immune serum to find new outer membrane protein antigens identified 21 new antigens.
- The genomic approach using protein expression from genes predicted to encode outer membrane proteins also identified ~20 new antigens.
- These methods save time.
- These approaches enable us to begin to develop effective vaccines for very complicated pathogens.
- Saving the life of just one cow per family in many impoverished areas of the world would be a huge benefit for human health.

Questions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