

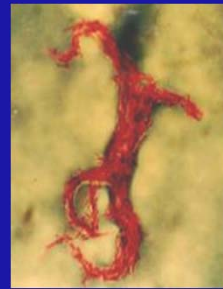
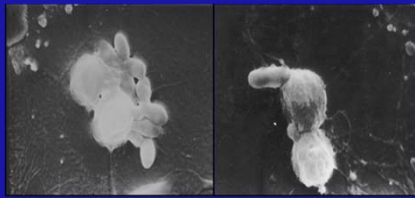
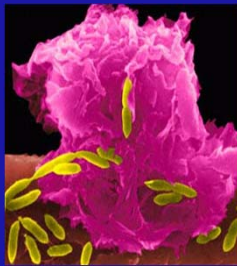
## TB Vaccines: Where Are We and What Needs To Be Done?

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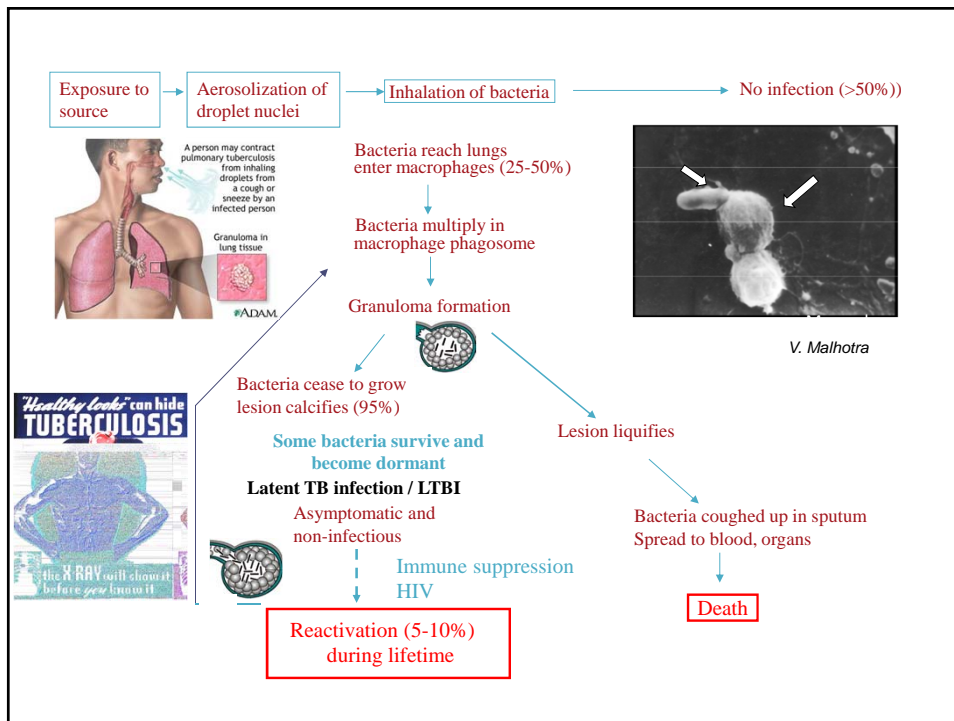
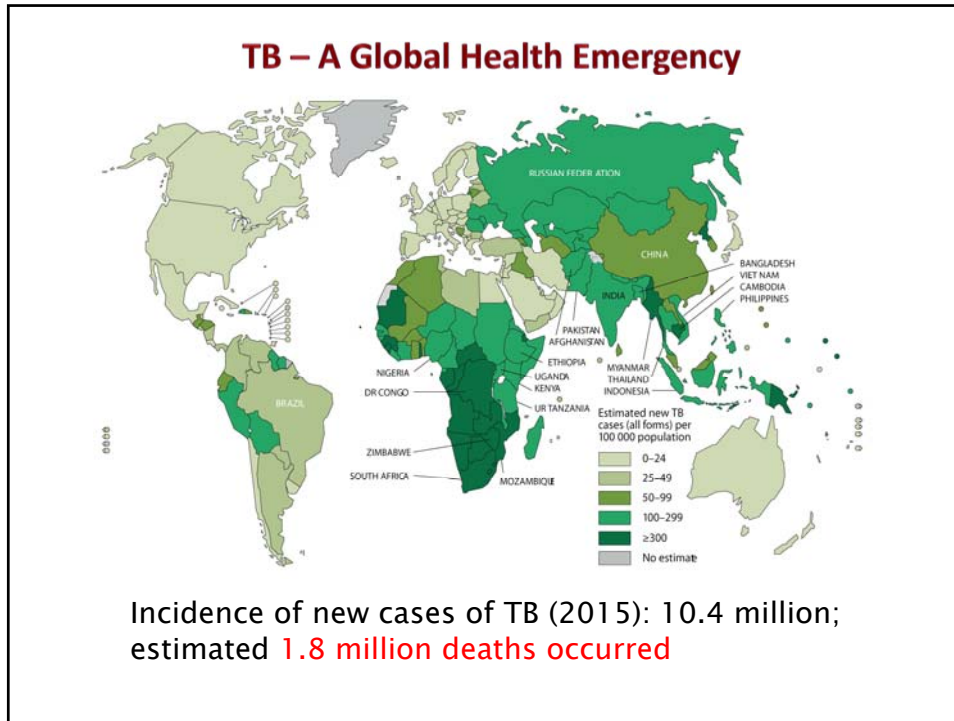
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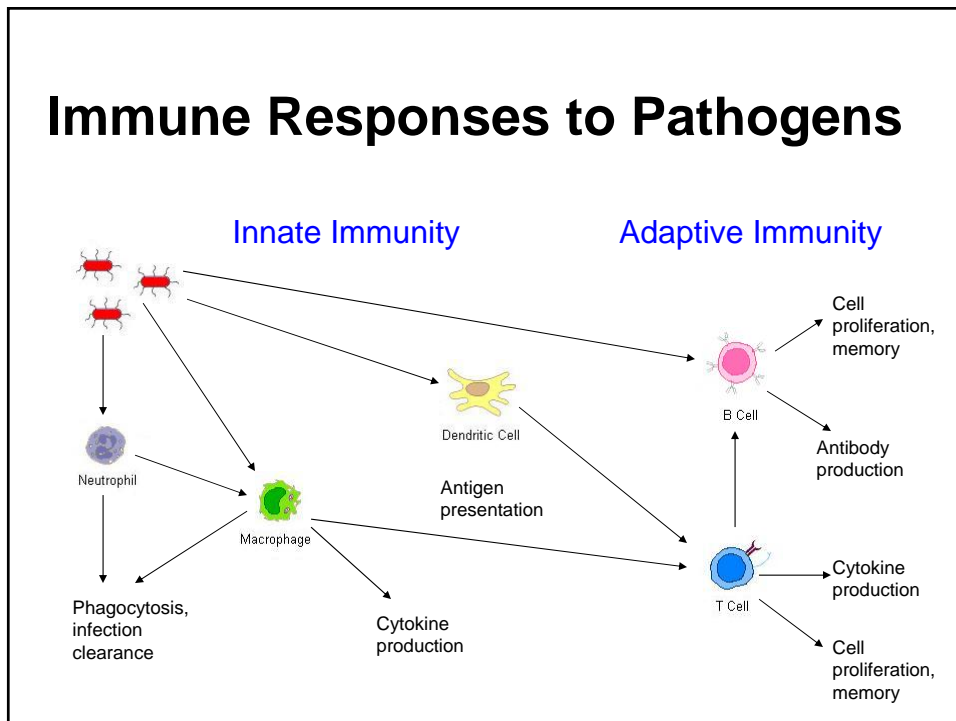
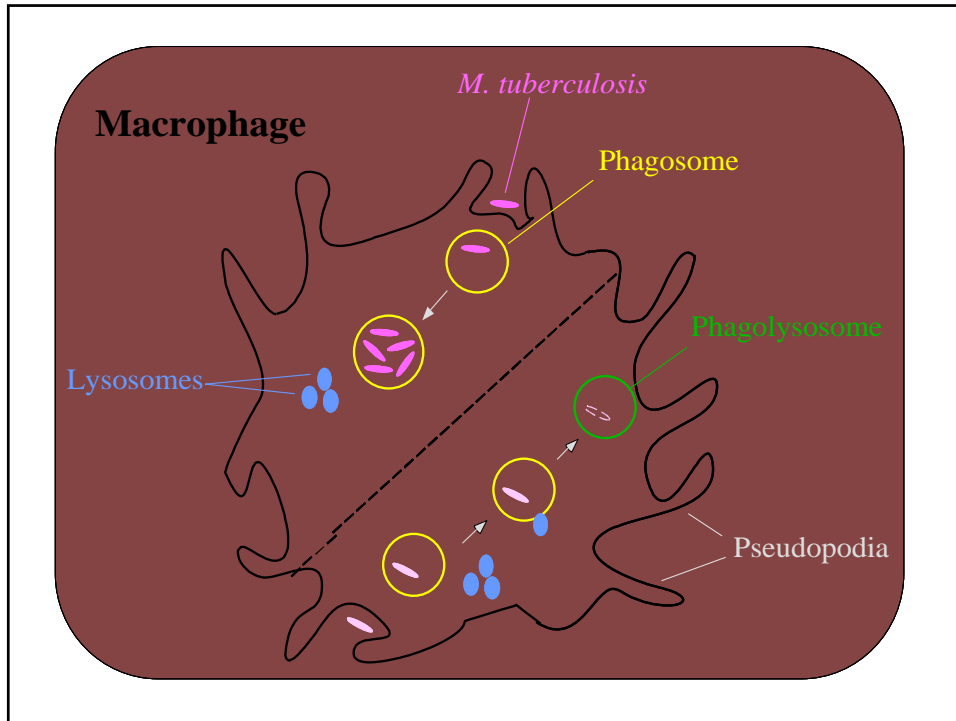
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### Overview

- *Mycobacterium tuberculosis* background
- TB vaccines currently under development
- Recombinant attenuated *Salmonella* vaccines (RASVs)
- Development of RASV-*M. tuberculosis* vaccine





## **Universal problems in generating completely safe but highly immunogenic attenuated vaccines**

- 1. All pathogens have devised means to subvert, suppress, modulate and/or evade host immune responses.**
- 2. Attenuation invariably reduces immunogenicity by reducing ability of vaccine to access and persist in lymphoid tissues.**

### **BCG Vaccine against *M. tuberculosis***

BCG = Bacille Calmette Guérin

BCG is a live bacterial vaccine: an attenuated strain of *Mycobacterium bovis*, which is closely related to *M. tuberculosis*

BCG is a safe vaccine: it is routinely administered to newborn babies in almost all countries in the world except western Europe and U.S.

Immunization with BCG protects babies and young children from many serious complications of TB, such as tuberculous meningitis and disseminated (miliary) TB

#### **BUT**

Protection against TB is not long-lasting: by the time children reach adolescence and young adulthood, they are susceptible to infection by *M. tuberculosis*

## TB Vaccines Under Development

- **Whole cell vaccines:**
  - VPM1002: recombinant BCG producing listeriolysin
  - MTBVAC: attenuated *M. tuberculosis* ( $\Delta phoP \Delta fadD26$ )
  - *Mycobacterium vaccae*: heat-inactivated
  - DAR901: Heat-inactivated *M. obuense*

## TB Vaccines Under Development

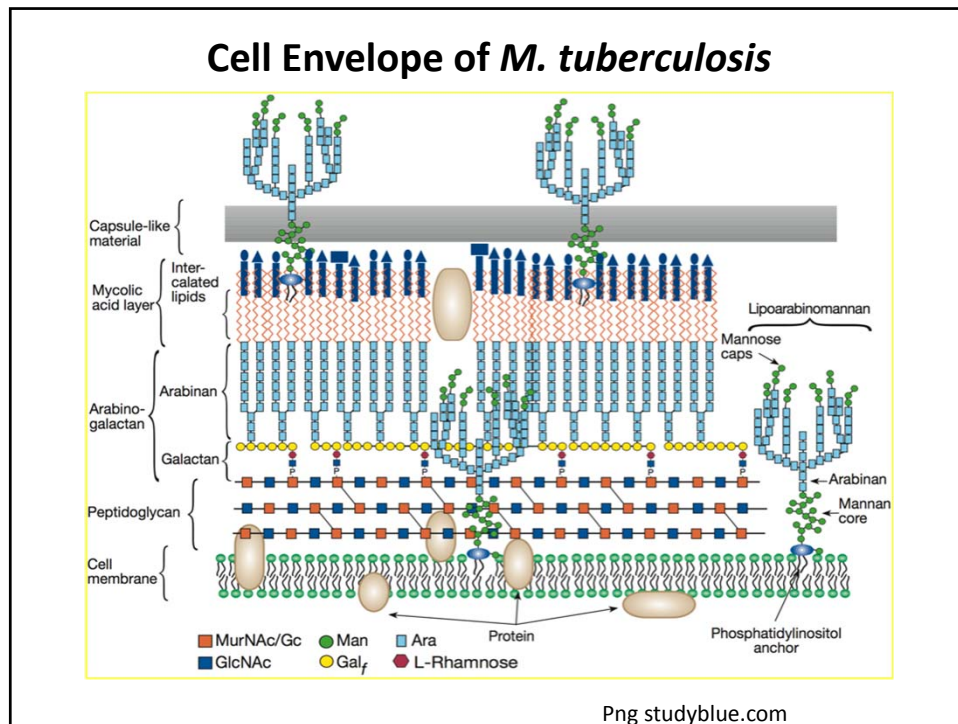
- **Viral vectored vaccines:**
  - MVA85A: Attenuated Vaccinia virus MVA producing Ag85A
  - Crucell Ad35/Aeras 402: Adenovirus 35 producing Ag85A, Ag85B, TB10.4
  - AdAg85A: Adenovirus 5 producing Ag85A
  - RhCMV (Rhesus cytomegalovirus) producing 6 *M. tuberculosis* antigens

### ***M. tuberculosis* Antigens Used in Vaccines**

- **ESAT-6 (*esxA*)**
  - Early secreted antigenic target; immunodominant T cell antigen; TB10.4 is a homologue
- **CFP-10 (*esxB*)**
  - Culture filtrate protein – 10 kDa; immunodominant T cell antigen, secreted with ESAT-6
- **Ag85A & Ag85B (*fbpA*, *fbpB*)**
  - Mycolyl transferases necessary for synthesis of trehalose dimycolate (mycolic acid)
- **Mtb72 (fusion of *mtb32* and *mtb39A*)**
  - PPE18, antigenic protein, unknown function

### **TB Vaccines Under Development**

- **Adjuvanted Subunit vaccines:**
  - **Hybrid-IC31: Ag85B + ESAT-6**
  - **Hybrid1-CAF01: Ag85B + ESAT-6**
  - **M72 + AS01E: Fusion of Mtb32A & Mtb39A to form M72**
  - **H4:IC31: Ag85B + TB10.4**
  - **H56:IC31: Ag85B + TB10.4 + Rv2660**
  - **ID93 + GLA-SE: Rv2608, Rv3619, RV3620 & Rv1813**



## Why use *Salmonella* as a vaccine?

### **Why *Salmonella* as a vaccine vector?**

Mucosally-delivered *Salmonella enterica* Serotypes efficiently attach to and invade mucosal associated lymphoid tissues (MALT, GALT, NALT, BALM, etc.) in birds, non-human mammals and humans before colonizing internal lymph nodes, liver and spleen.

All routes of delivery result in induction of memory for mucosal, systemic and cellular immune responses leading to long-term protective immunity.

We know a great deal about the pathogenicity, physiology, genetics and genomics of *Salmonella* serotypes and *Salmonella* can be genetically manipulated with ease.

### **Objectives for Orally Delivered Live *Salmonella* Vectored Vaccines**

- Maximize invasiveness to and colonization of internal effector lymphoid tissues
- Recruit innate immune responses without being over reactogenic to cause distress or disease symptoms
- Maximize induction of mucosal, systemic and cellular immunities
- Be safe for newborn, pregnant, malnourished and immunocompromised individuals
- Exhibit biological containment



### **We achieve our objectives by genetically modifying *Salmonella* to exhibit:**

1. Regulated delayed in vivo expression of attenuating phenotype
2. Regulated delayed in vivo expression of codon-optimized sequences encoding protective antigens to be delivered by secretion and/or lysis in vivo
3. Regulated delayed in vivo lysis to deliver a bolus of protective antigens or DNA vaccine, which maximizes innate and adaptive immune responses and confers complete biological containment

**These three features enable the vaccine strain at the time of oral vaccination to exhibit the same or better attributes than the wild-type parental strain to survive host-induced stresses to increase ability to colonize internal lymphoid tissues to maximize immunogenicity.**

### **We further achieve our objectives by genetically modifying *Salmonella* to exhibit:**

4. Constitutive expression of a highly invasive phenotype to enhance colonization of lymphoid tissues to induce maximal levels of protective immunity

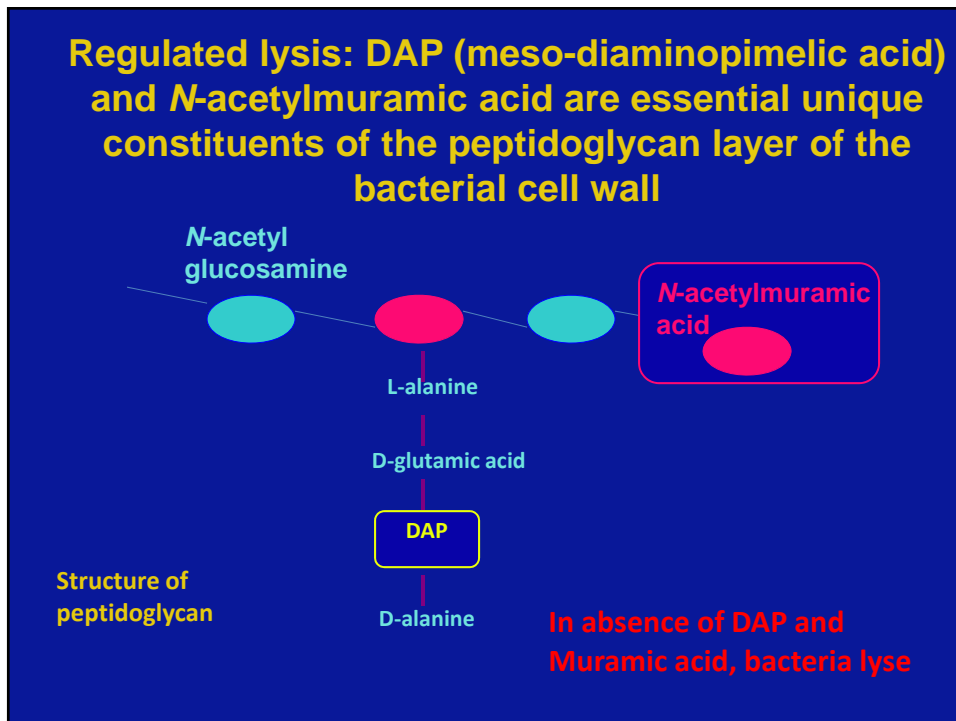
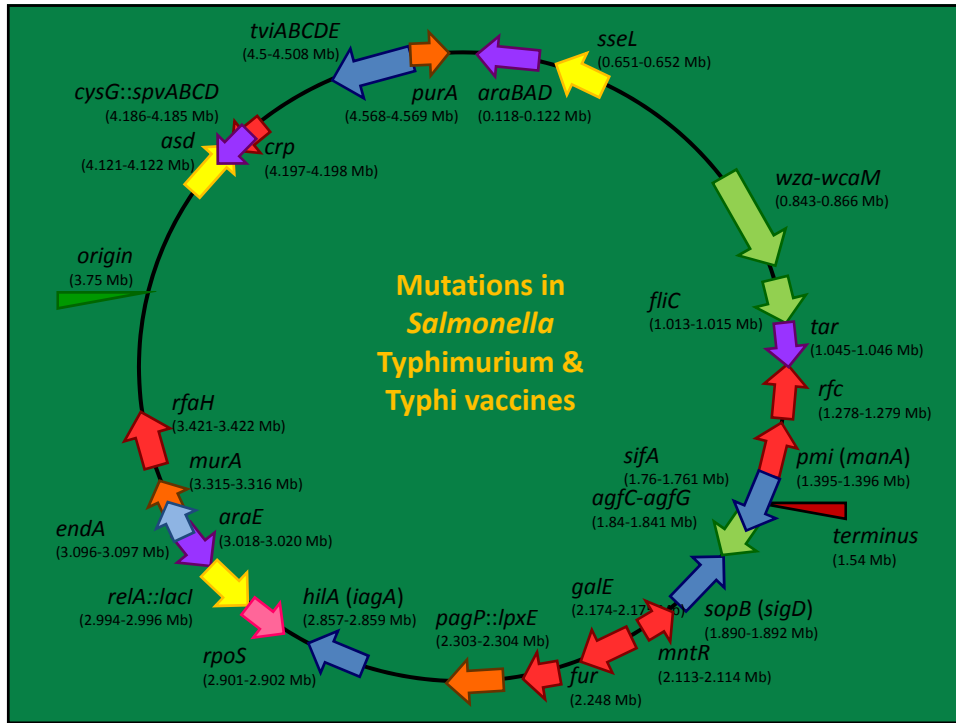
**This decreases dose of vaccine needed to induce protective immunity**

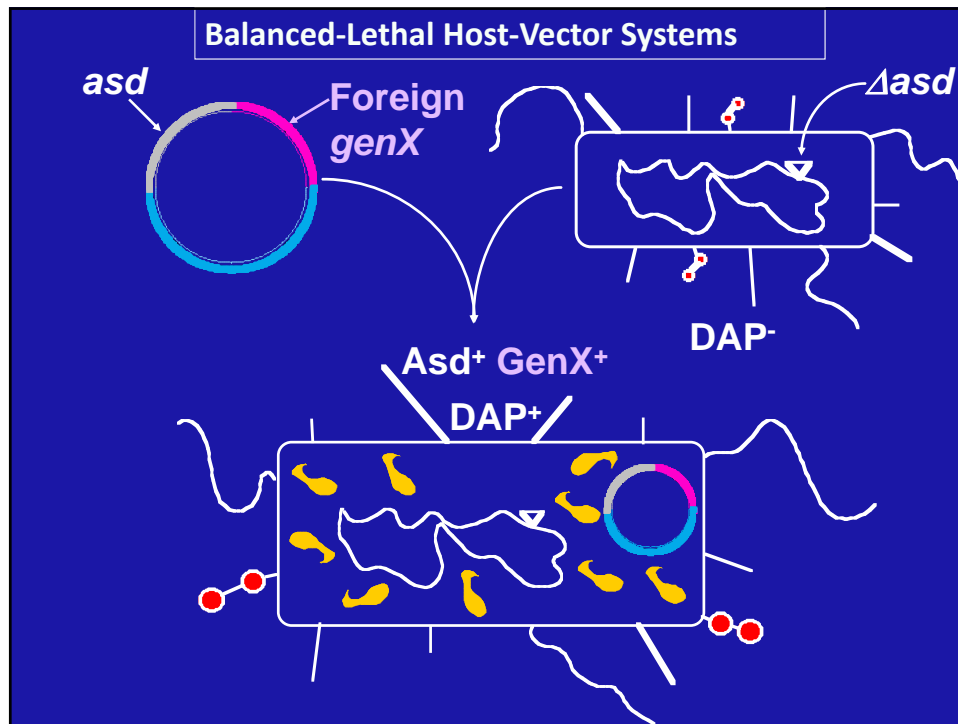
5. Inability to synthesize multiple surface-associated carbohydrate and protein polymers to preclude ability to form biofilms

**This blocks persistence on gall stones and in intestines and facilitates lysis of strains with the regulated delayed lysis in vivo phenotype**

6. Decreased ability to suppress or modulate induction of immunity

**These multiple alterations all act to enhance & maximize immunity**





### Antigen Delivery Strategies by Recombinant Attenuated *Salmonella* Vaccines (RASVs)

- within cytoplasm of *Salmonella* vaccine
- by partial secretion using the Type 2  $\beta$ -lactamase (or other) secretion signals and by enhancing production of outer membrane vesicles
- by secretion/translocation via a Type 3 secretion system
- by surface presentation using a Type 5 secretion system
- release by lysis of *Salmonella* in vivo

**Live Recombinant *Salmonella*  
Vaccines Against *Mycobacterium  
tuberculosis***

**RASV Strain**

- The recombinant attenuated *Salmonella* vaccine (RASV) strain used has multiple deletion mutations and deletion-insertion mutations to result in regulated delayed lysis of the strain in vivo.
- Regulation by arabinose:
  - Arabinose present: genes are transcribed
  - Arabinose absent: no transcription; essential proteins are not produced → RASVs lyse

## Biological Containment of Vaccine Strains by In Vivo Lysis

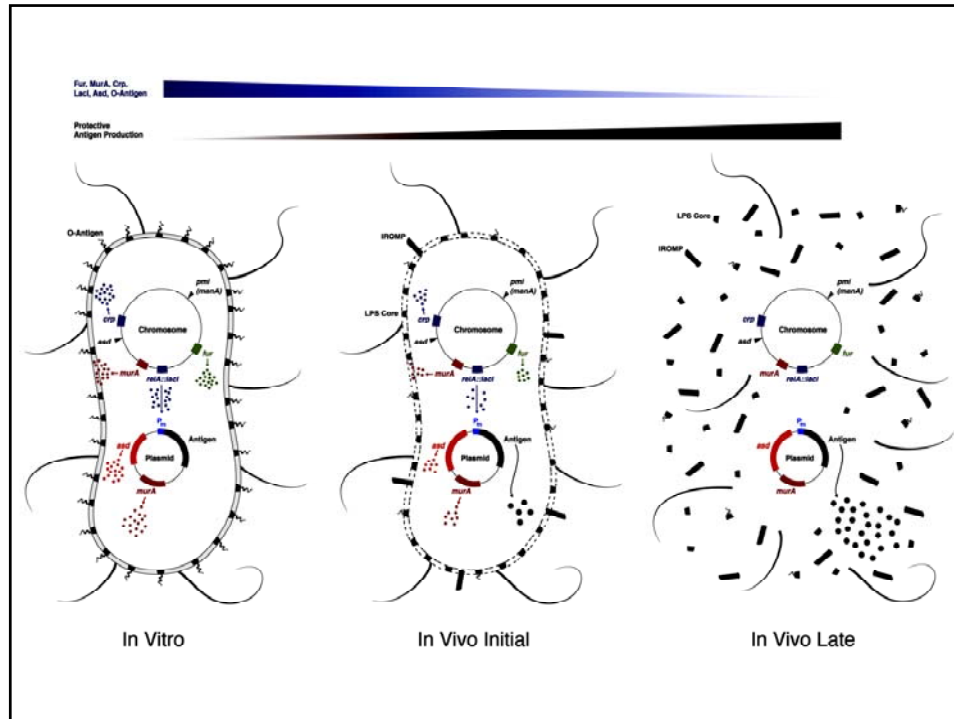
Achieved by regulated expression of essential genes for synthesis of muramic acid and diaminopimelic acid (DAP)

- Is a type of regulated delayed attenuation
- Results in no vaccine strain persistence in vivo and no survivors if excreted
- Can be used to deliver a bolus of protective antigen or a DNA vaccine vector

### DAP-less and Muramic-less Death in Host Strain with Regulated Lysis System Vector



Recombinant host-vector strain displaying arabinose-dependent growth and regulated cell lysis in the absence of arabinose.



### RASV-*M. tuberculosis* Vaccines

**Goal:** To deliver protein antigens of *M. tuberculosis* to the cytoplasm of eukaryotic cells in order to elicit a cell-mediated immune response.

- *M. tuberculosis* protein antigens known to elicit protective T-cell responses (ESAT-6, CFP-10, Ag85A) were delivered by attenuated *Salmonella* vaccine strains by three mechanisms:

(1) As fusion proteins with (a) *Salmonella* Type III Secretion System effector protein SopE2 and (b) Type II Secretion signal sequences from  $\beta$ -lactamase or OmpC.

(2) By delivery of the antigens into the eukaryotic cell cytoplasm following escape from the endosome and regulated delayed lysis of the RASV strain.

## *Mycobacterium tuberculosis* Antigens for Vaccine Development

- |                           |   |
|---------------------------|---|
| <b>ESAT-6</b> <i>esxA</i> | Early secreted antigenic target of <i>M. tuberculosis</i> ; immunodominant T cell antigen   |
| <b>CFP-10</b> <i>esxB</i> | Culture filtrate protein - 10 kDa; co-transcribed with <i>esxA</i> ; proteins probably secreted together; immunodominant T cell antigen |
| <b>Ag85A</b> <i>fbpA</i>  | Mycolyl transferase, involved in the synthesis of trehalose dimycolate, one of the mycolic acids  |

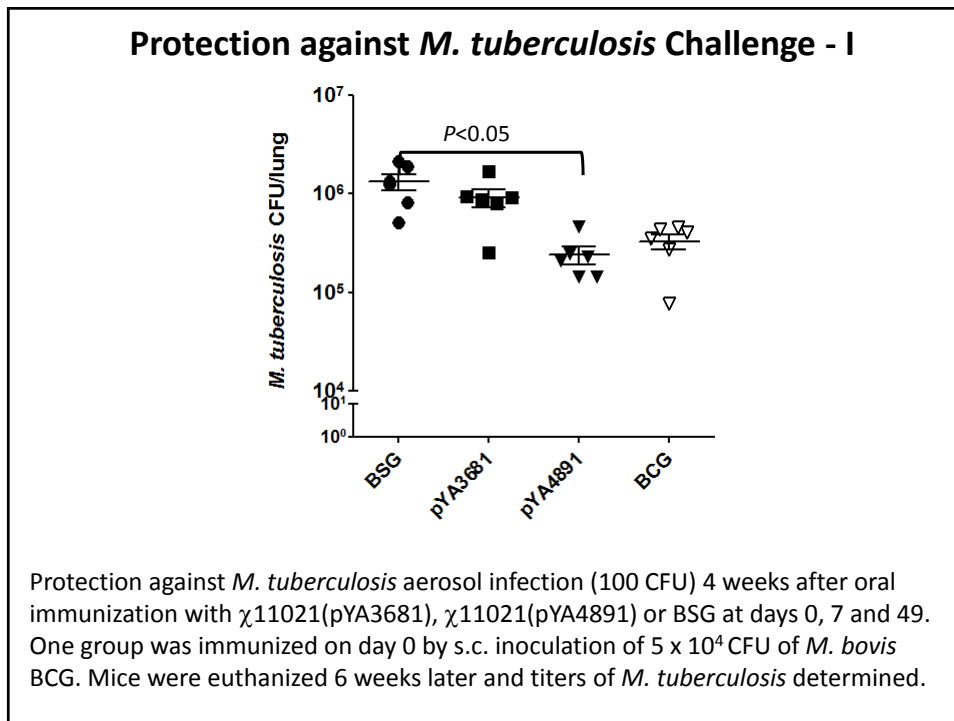
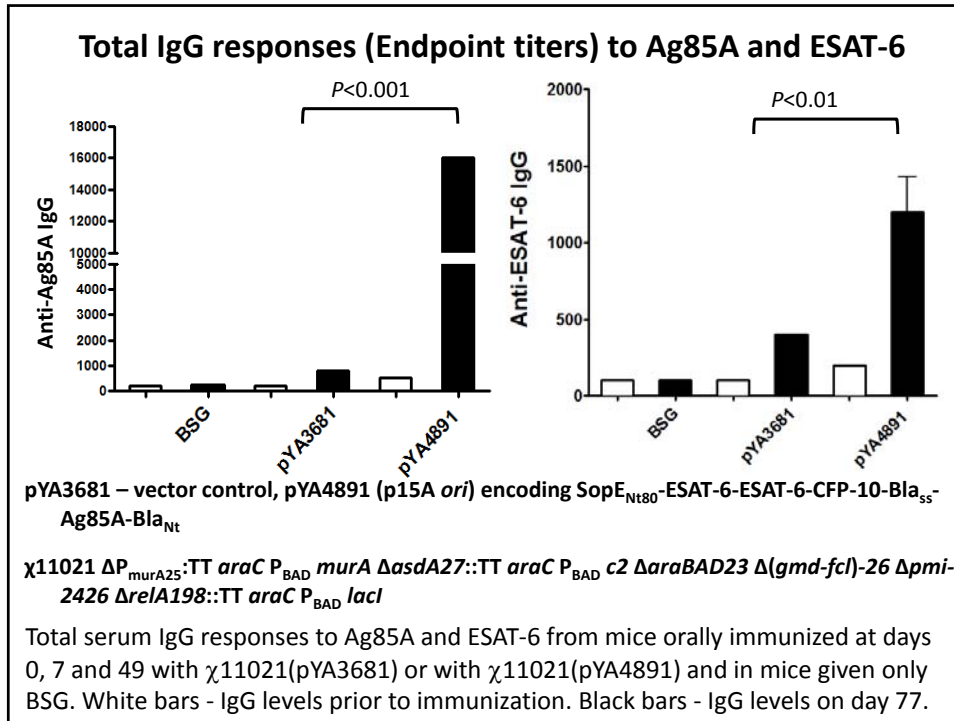
### Plasmids specifying synthesis of *M. tuberculosis* antigens in RASVs

**SopE<sub>Nt80</sub>-ESAT-6-ESAT-6-CFP-10-Bla<sub>SS</sub>-Ag85A-Bla<sub>CT</sub>:**

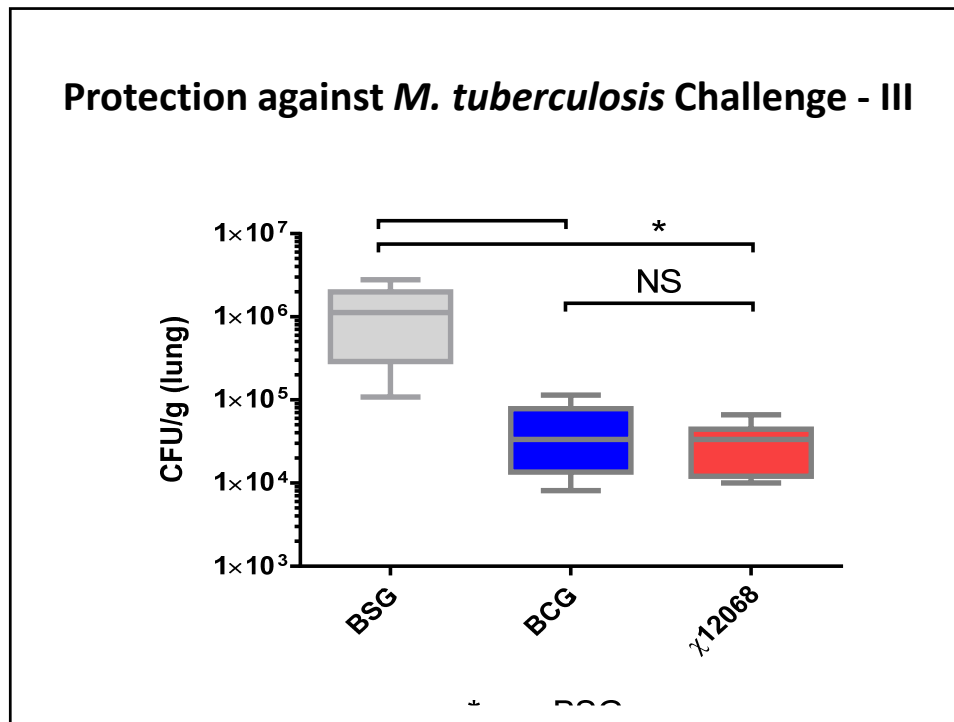
**pYA4890 (pBR *ori*)**

**pYA4891 (p15A *ori*)**

**pYA4892 (pSC101 *ori*)**







### Summary

- Live recombinant attenuated *Salmonella* vaccine (RASV) strains with regulated delayed lysis in vivo produce, secrete & release *M. tuberculosis* antigens.
- RASV-*M. tuberculosis* vaccines elicit antigen-specific antibody and cytokine responses, suggestive of a Th1 immune response.
- RASV-*M. tuberculosis* vaccines elicit protection in immunized mice against aerosol challenge with live *M. tuberculosis* H37Rv that is similar to or better than protection by *M. bovis* BCG vaccination.

## Current RASV-Mtb Research

- We have eight additional protective or putative protective Mtb antigens (11 total) to be delivered by our most recently improved RASV vector strain with regulated in vivo lysis.
- Each is being evaluated alone and in operon fusions of two to three antigen coding sequences.
- We are also evaluating immunizing with mixtures of RASVs delivering multiple protective Mtb antigens.

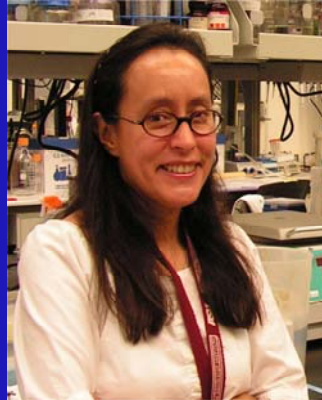
## Additional *M. tuberculosis* Antigens

- **FAP/Apa** – Immunogenic 45 kDa protein
- **TB15.3** – Iron-regulated immunogenic protein
- Resuscitation factors – **RpfA, RpfB**
- **GlnA1** – Glutamine synthetase
- **Mpt63** – Secreted immunogenic protein
- **EspC** – associated with Esx-1 secretion system
- **TB10.4 & TB9.8** – Homologues of ESAT-6 and CFP-10

## **RASV: *M. tuberculosis* Vaccine Team**



*Jiseon Yang*



*Maria Dolores Juarez-Rodriguez*



*Lourdes Arteaga-C*

## **RASV-*M. tuberculosis* Vaccine Team, cont.**

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- Megan Caraway
  
- Shilpa Sanapala
- Shifeng Wang
- Kit Dkhar
- Christie Hay

**Research supported by**

