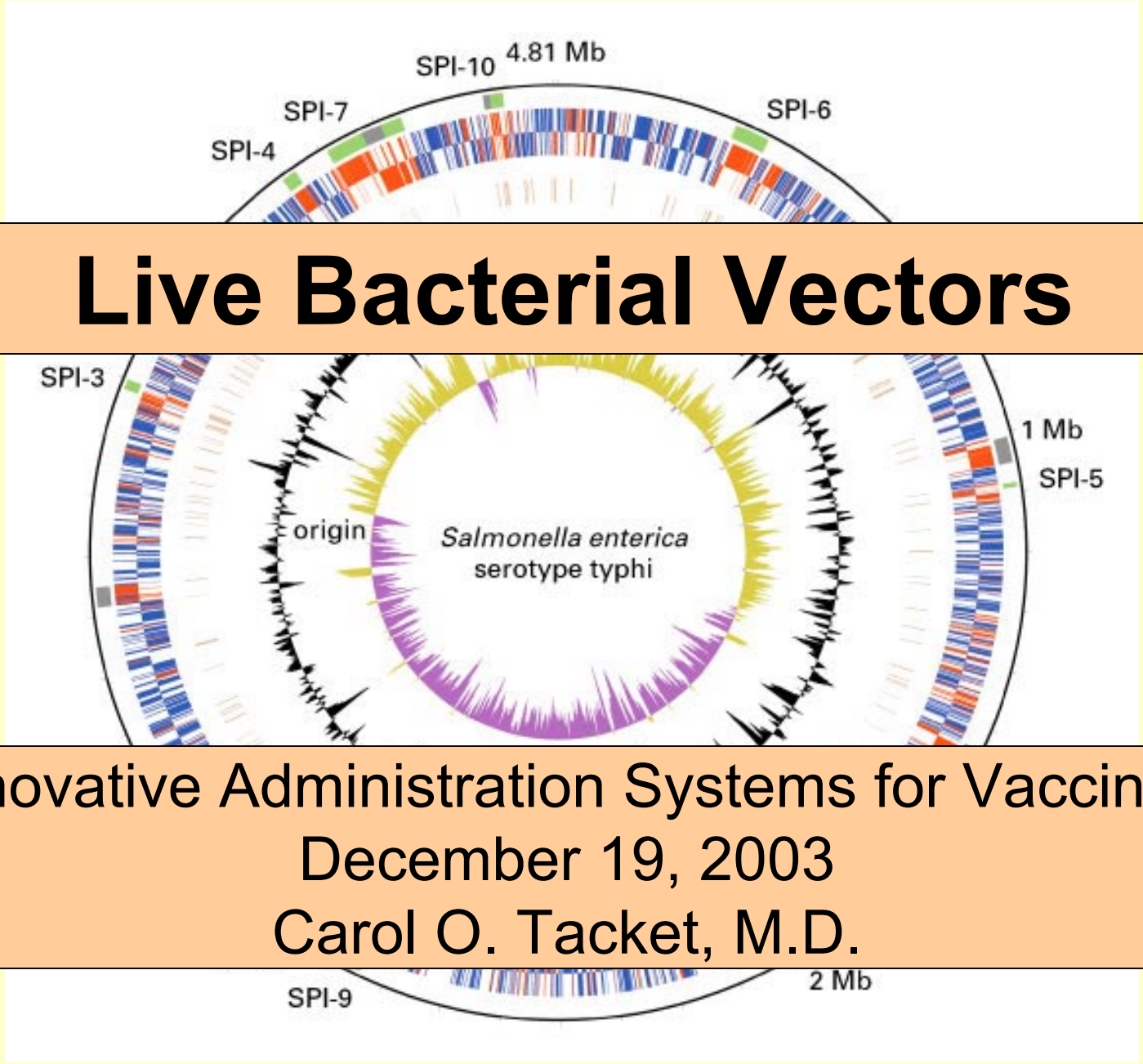
Live Bacterial Vectors



Innovative Administration Systems for Vaccines
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Types of Live Bacterial Vectors

- Those based on commensal bacteria
  - e.g., Lactobacilli
- Those based on attenuated pathogens
  - e.g., Salmonella, Shigella, BCG, Listeria

Salmonella enterica serovar Typhi vectors

- Bacteria express vaccine antigen by prokaryotic expression plasmids

or

- Bacteria deliver foreign genes carried on eukaryotic expression systems (DNA vaccines)

Pathogenesis of Typhoid Fever

7-14 days

- Oral ingestion of *S. enterica* serotype Typhi
- Passage through the gastric acid barrier
- Mucosal attachment and internalization via M cells overlying Peyer's patches
- Translocation to lymphoid follicles and mesenteric lymph nodes
- Primary (silent) bacteremia
- Seeding of liver, spleen, lymph nodes, gall bladder
- End of incubation period
- Secondary bacteremia and symptom onset

Putative Protective Immune Responses Elicited by *S.* Typhi Vector Antigens

- **Mucosal IgA LPS O and flagellar H antibodies**
- **Serum LPS O and H antibodies**
- **Cell-mediated immunity**:
  - **Cytokine-producing (IFN-γ) proliferative lymphocytes**
  - **CTLs**

Can analogous responses be elicited to the vectored antigen?

Licensed Ty21a is pretty good, but neither an ideal typhoid vaccine nor an ideal vaccine vector.

- Requires multiple doses for maximum immune response against *S*. Typhi
- Basis for attenuation is unknown.
- Clinical studies generally unsuccessful
  - Ty21a-*Shigella sonnei* O polysaccharide gave inconsistent protection against shigellosis.
  - Ty21a-*V. cholerae* Inaba LPS induced only modest rate of response to LPS.
  - Ty21a-*H. pylori* urease-weak T cells responses, no humoral response to urease.

Start over with wild-type *S.* Typhi Strain

Gene	Attenuating phenotype
aro	**Dependence on nutrients not available in human host**
cya crp	**Deletion of global regulatory system**
phoP/phoQ	**Loss of response to environmental signals**
htrA	**Decreased ability to survive in macrophages**
cdt	**Interfere with ability to invade deep tissues**
ssaV	**Interrupt type III secretion system**
Combinations	

Animal model
S. Typhi is restricted to humans

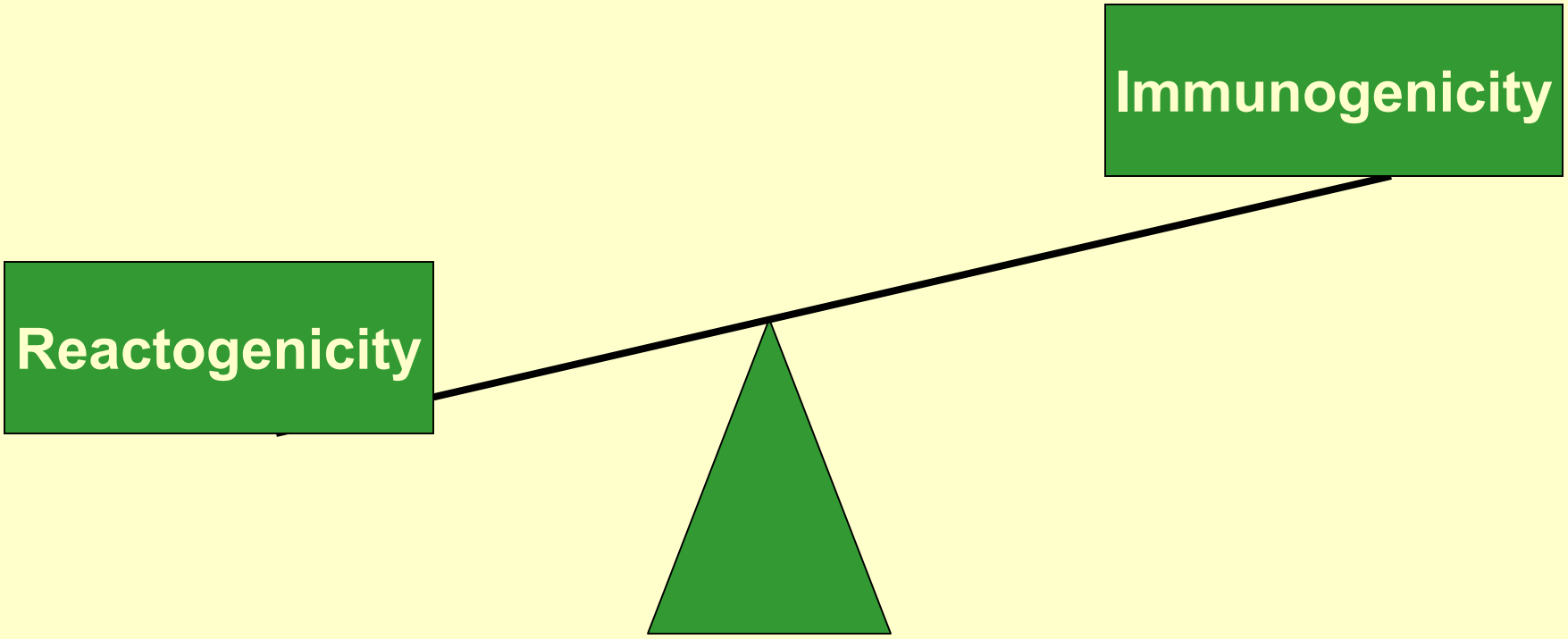
- **Murine typhoid**
  - **Oral infection of mice with *S.* Typhimurium or *S.* Enteriditis**
- **I.p. *S.* Typhi adsorbed to hog gastric mucin**
  - ***S.* Typhi infect and survive within peritoneal phagocytic cells.**
  - **Murine LD_{50} of 10^5**
  - **Mice survive about 48 hours.**

Pre-clinical studies of *S.* Typhi vaccine strains in mice have not always correlated with the results of clinical trials.

Alternative animal model

- Murine intranasal model for assessing immunogenicity of *S*. Typhi strains
  - NALT induces responses to vector and heterologous antigen
  - Cell mediated and serologic responses

A Not-So-Delicate Imbalance



Ty800
Deleted in *phoP phoQ*

- Well tolerated in 11 volunteers in doses up to 10^{10} cfu
- No vaccine bacteremia
- Good immune responses

Hohmann et al. J Infect Dis 1996;173:1408.

CVD 908-*htrA*
Deleted in *aroC aroD htrA*

- No increased incidence of fever or diarrhea compared to placebo recipients
- No positive blood cultures
- Minimal fecal shedding (up to 3 days)
- Good immune responses
  - Antibody secreting cells
  - Serum antibody
  - Cell-mediated immunity

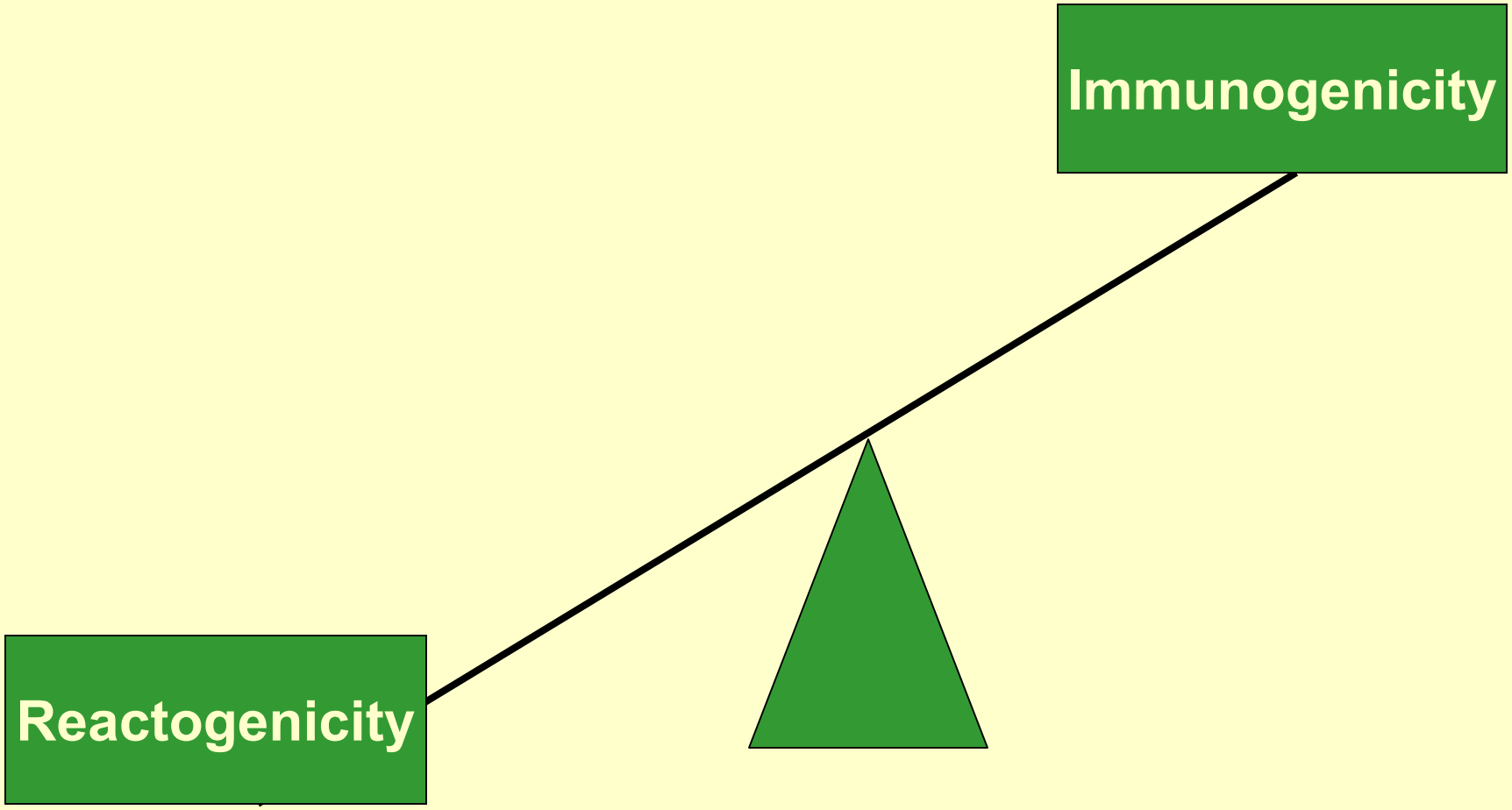
Tacket et al. Infect Immun 1997;65:452.

ZH9
Deleted in *aroC* and *ssaV*

- Single dose of 10^7-10^9 well tolerated in 9 volunteers
- No bacteremia
- Brief shedding
- Good immune responses at the higher doses

Hindle et al. Infect Immun 2002;70:3457.

The Optimal Balance



Antigens Expressed in Attenuated Salmonella Vectors in Humans

- **Tetanus toxin fragment C**
- **Circumsporozoite protein (CSP) of *P. falciparum***
- **Hepatitis B core and pre-S**
- ***H. pylori* urease**

CVD 908-htrA(pTETlpp)-tetanus toxin fragment C

- 9 healthy adult volunteers
- Single dose of 10^{8-9} cfu
- No fever or bacteremia; some mild diarrhea and vomiting
- Decreased responses to *S*. Typhi LPS and H responses
- 3/9 who received 10^{8} cfu or more developed rises in serum antitoxin antibodies.

Tacket et al. Clin Immunol 2000;96:146.

CVD 908-rCSP

- 10 volunteers
- Two doses of $5x10^8$, 8 days apart
- Strong *S*. Typhi LPS and H responses
- 2/10 had rises in serum anti-CSP
- 1/10 had CSP-specific CD8+ CTL

Gonzalez et al. J Infect Dis 1994;169:927.

χ4632(pYA3167)-HBc-pre-S fusion

- 10 volunteers
- Single oral dose of 3×10^7 or 7×10^8
- Good responses to *S*. Typhi antigens at the higher doses
- No serum antibody to hepatitis pre-S or pre-S-specific ASC

Tacket et al. Infect immun 1997;65;3381.

Ty1033-urease

- 8 volunteers
- Single or double dose of 10^{10} cfu
- Strong *S*. Typhi LPS and H responses
- None of 8 had immune response to urease
- None of 3 had anti-urease response after oral booster of rUrease and LTB

DiPetrillo et al. Vaccine 1999;18:449.

Room for Improvement

- Selection of adequate plasmids and promoters to reduce metabolic burden to host bacteria
- Stabilization systems to allow adequate antigen expression
- Control of site of expression of antigen, i.e., cytoplasm or extracellular
- Preservation of conformation of antigen epitopes

Salmonella-vectored Vaccines for Rapid Deployment to Large Populations

- Yersinia pestis
- Anthrax
- Botulinum toxin

- ?part of a prime-boost strategy with parenteral vaccine prime

Rapid deployment to large populations

- Ease of administration
- No needles/syringes
- No risk of transmission of human pathogens
- Mucosal immune responses; protect against infection as well as disease?
- Multiple antigens in single strain
- Co-expression of immunomodulators (cytokines or adhesins)
- Public acceptance

- Cold chain requirement
- Need to monitor ingestion of whole dose of liquid formulation (esp. children)
- Probably unsafe for immunocompromised individuals
- Currently no clinical tests of Salmonella-based vaccine against agent of bioterror

Summary/Conclusions

- Several safe *S*. Typhi vectors identified in preliminary clinical studies
- Modest responses to foreign antigens in clinical studies of several *S*. Typhi vector strains
- Many possible strategies for improving immunogenicity