The Creation of a Vaccine for Porcine Reproductive and Respiratory Syndrome Virus

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Purpose and Goals

- Create an effective vaccine against PRRSV
- Currently no effective vaccines
- The virus infection costs $560 million annually in the United States pork industry

Cho et al. 2006
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

- Isolated in 1990 in Netherlands
- Persists in tissue for approximately 150 days
- Younger pigs have longer infections
- Highly contagious

Effects of the Virus

- Growth reduction and heightened mortality
- In sow, reduction in conception rates, birth of infected piglets
- In boar, loss of libido, lethargy, alterations in seminal quality
- Clinical effects may depend on strain

**Bacillus Bacteria**

- Aerobic, Gram positive, rod-shaped
- *B. anthracis* and *B. thuringiensis*
- Produce spores
- Use of spores in vaccines
- Non-pathogenic strains were used

**Bacillus Endospore Antigen Display System**

- Hair-like projections
- Fusion of foreign proteins onto spore
- Basis of the BEADS platform
- Express PRRSV viral protein on outside of spores

Thompson et al. 2008
Methods

- Vaccine Production
- Sera Production
- Protein Production
- Western blotting
Vaccine Production

- Created the *bclA-orf5* recombinant gene
- Inserted into *B. thuringiensis*
- Grown on nutrient agar plates until sporulation
- Spores were collected and killed
Vaccine Inoculation and Sera Extraction

- Vaccinated three sets of mice
- Set 1: killed virus coated spores
- Set 2: killed normal spores
- Set 3: Saline control
- Sera were extracted from each set after 6 weeks
- Used in Western blots
Protein Production

- The orf5 gene cloned an *E. coli* plasmid and transformed into *E. coli*
- Recombinant protein released by cell lysis
- Purified via affinity column
- Used in western blots
Western blots

- To test effectiveness of the vaccine
- Used to detect presence of certain protein
- Standard Western blot procedure
- Incubated with sera
- Developed a film image

From www.tata-box.com
To test expression of PRRSV viral protein on the spores
- Only exposed pigs would interact with the spores
- No cross reactivity between immune and preimmune sera
- Proper expression of PRRSV viral protein
- Proper vaccine delivery vehicle
Western blot

- To verify the spores coated with viral protein
- All proteins on the spore separated by size
- 190 kDa: large complexes from spores
- 60 kDa: BclA fused with Viral protein

Lane 1: wildtype B. t. spores
Lane 2: PRRSV ORF5 coated B. t. spores
Western blot – Pig

- To test whether viral protein can be made in *E. coli*
- Primary: Pig immune sera
- Remnants of non-specific protein
- Recombinant protein recognized by pig antibodies
Prove viral protein will not interact with naïve mice sera
Primary: Mouse PBS control sera
Naïve mice do not have antibodies towards PRRSV
Band is non-specific
Same results seen with spore only vaccinated group
Test the effectiveness of the vaccine
- Primary: Mouse immune sera from ORF5 tagged spore vaccination group
- rPRRSV-ORF5 protein
- Proves 30 kDa is nonspecific and 28-29 kDa band is due to antibody response
Future Direction

- Refinements necessary
- Faintness of 28-29 kDa band
- Increasing expression of viral protein on the spore
- **Field testing**
  - Vaccinate pigs, collect sera
  - Western blotting
- Expansion of BEADS Platform
Bibliography

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Acknowledgements

• Mentors: Dr. Brian Thompson and Dr. Hsin-Yeh Hsieh (University of Missouri)
• Dr. George Stewart
• Teachers: Mr. Inglis, Ms. Dyer, Dr. Cohen
• Parents: Benjamin and Shuyi Hsing