

Experiment Number: 5438-32000-024-04

ROMAN L. HRUSKA U.S. MEAT ANIMAL RESEARCH CENTER

Experimental Outline

1. Project Title and Number:

Host Response and Antigenic Variation of Respiratory Viral Agents of Livestock (5438-32000-024-00D)

1a. Experiment Title:

Identification of Responsive Genes in Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)-infected Swine Pulmonary Alveolar Macrophages using Serial Analysis of Gene Expression (SAGE).

2. Experiment Leaders:

Laura C. Miller

3. Person Responsible for Analysis of Data and Publication of Results:

Laura C. Miller

4. Research Unit Involved:

Animal Health (AH)

5. Specific Experimental Objective:

To identify host genes which are differentially regulated as a result of PRRSV replication in pulmonary alveolar macrophages.

6. Rationale for the Experiment:

Porcine reproductive and respiratory syndrome (PRRS) is a disease found on swine farms world wide and is characterized by reproductive failure in late-term pregnant sows, and respiratory illness and mortality in young pigs (1). Attempts to develop vaccines against PRRSV have been met with limited success (reviewed in (2)). Thus, PRRS remains a serious problem for swine producers. Very limited information is available in terms of the animal host response mounted against PRRS during an infection. Studies published indicate that both a cell mediated and antibody mediated host response are necessary to clear PRRSV infection (3). Gaining insight into the mechanisms of viral clearance at the gene

expression level would undoubtedly provide clues into methods for better developing more effective vaccines. The objective of this experiment is to identify porcine alveolar macrophage genes that are differentially regulated as a result of PRRSV infection.

Literature Cited

1. Goyal, S. M. (1993). *J Vet Diagn Invest* 5(4): 656-664.
2. Dee, S. A., H. S. Joo, D. D. Polson, & W. E. Marsh. (1997). *Vet Rec* 140(19): 498-500.
3. Bautista, E. M. and T. W. Molitor. (1997). *Viral Immunol* 10(2): 83-94.

7. Specific Experimental Procedures:

Initially, three healthy pigs, 8 weeks in age, will be utilized to optimize macrophage storage, infection conditions, and RT-PCR template quality of RNA extracted. Further pigs will then be utilized after conditions have been optimized. Animals currently housed at MARC will be delivered to the GPVEC necropsy suite the day of utilization. The pigs will be sacrificed by intra-cardiac injection of Beuthanasia-D under the supervision of Shuna Jones and Will Laegreid. Immediately following death the lungs will be removed and lavaged to isolate the pulmonary alveolar macrophages (PAM's). The PAM's will be enriched by removal of red blood cells and monocytes using red blood cell lysis buffer and several centrifugal pelleting steps. Cryopreservation conditions of PAM's will be optimized in the initial experiment. Also, culture of PAM's and infection with PRRSV will be optimizing by comparison in a non-adherent state and plated state. The PAM's will be plated in tissue culture flasks and upon adhesion to the plate surface (1 hour), will be washed further to remove the majority of remaining monocytes.

The PAM's will be cultured for 24 hours at which time they will be divided into the following groups: A) 0-hour control, B) 4-hour PRRSV infected, C) 12-hour PRRSV infected, D) 24-hour control, and E) 24-hour PRRSV infected. The control groups will be treated exactly as the PRRSV infected groups without the addition of PRRSV. The PRRSV infected cells will be infected with a multiplicity of infection of 10 and allowed to adhere at 4°C for 1 hour. The cells will then be shifted to 37°C and this will represent time 0. Thus, 4, 12 and 24 hours after time 0, cells will be harvested by scraping and suspension in RNA lysis buffer. The RNA will be purified and frozen under ethanol until further processing. A flask from each of the 3 animals will be utilized for each time point to eliminate any possibility of individual animal variability. After RNA is purified from each animal flask at a given time point, the RNA's will be pooled, sent to NADC to be used to generate an SAGE template. The PAM/PRRSV RNA will be compared relative to the equivalent PAM/control time point to eliminate variability due to length of culturing.

8. **Duration of Experiment:**

- A. Initiation Date: March 2006
- B. Completion Date: September 2006

9. **Specific Requirements of Research Unit:**

- A. SY's: 0.33 FTE for the experiment leader
- B. Support Services: None
- C. Equipment: Available in AH laboratories
- D. Supplies: Available in AH laboratories
- E. Data Processing Requirements: None
- F. Publication Costs: None
- G. Miscellaneous: None

10. **Specific Requirements of Livestock Operations:**

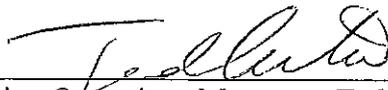
- A. Support Services: None
- B. Facilities: Use of GPVEC necropsy suite
- C. Livestock: A maximum of 6 pigs under the age of 9 weeks
- D. Feed: None
- E. Supplies: None
- F. Equipment: None
- G. Miscellaneous: None

11. **Estimated Receipts from Sale of Experimental Animals or Products:**

A maximum of 6 less pigs will be available for sale purposes. Given that they will be taken prior to 9 weeks of age, there will be minimal cost invested in production.

12. Reviewed by:

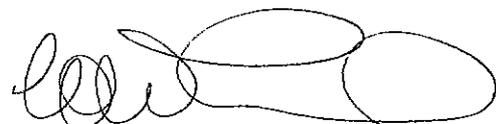
3/9/06  3/9/06
Date Rec'd CRIS Leader & Research Leader, Animal Health: Date
William W. Laegreid

3-9-06  3-9-06
Date Rec'd Swine Operations Manager: Ted W. Acton Date

Adequate resources (animals, labor, equipment) are available to conduct this experiment, it is experimentally sound, and it complies with the Roman L. Hruska U.S. Meat Animal Research Center's Animal Care Guidelines.

13. Approved by:

3/9/06  3/9/06
Date Rec'd Herd Health Veterinarian: Shuna Jones Date

3/9/06  3/9/06
Date Rec'd Research Leader, Animal Health: Date
William W. Laegreid

3/10/06  3/10/06
Date Rec'd Center Director: Mohammad Koohmaraie Date