RESEARCH ABSTRACT

SWINE HEALTH

Title: "Comparison of early immune responses of pigs which are genetically PRRS resistant/tolerant using a swine-specific immune protein (cytokine) multiplex assay." – **NPB #09-244**

Investigator: Joan K. Lunney

Institution: USDA, ARS, BARC, APDL, Beltsville, MD (BARC)

Co-Investigators: Jane Christopher-Hennings, Eric Nelson, Ying Fang, South Dakota State University (SDSU), Juan Pedro Steibel, Michigan State University (MSU), Jeffrey J. Zimmerman, Iowa State University (ISU)

Date Submitted: March 27, 2012

Scientific Abstract:

This NPB grant #09-244 has enabled our team to improve the Fluorescent Microbead ImmunoAssay (FMIA), the SDSU BARC FMIA, that was developed with NPB grant #08-189 (Lawson et al. 2010). Overall the use of the FMIA has enabled us to detect immune changes related to PRRSV infection using multiplex assays. This allows for uniform testing of multiple immune proteins (cytokines, chemokines) simultaneously within a small volume of sample and with a broader dynamic range for detection. Compared to past tests using multiple ELISA assays, the multiplex assay is less labor intensive, requires less sample and fewer replicates for each sample, and produces data in a shorter time due to high throughput analytic systems. Our tests have affirmed that cytokine protein levels in culture supernatants of cells, e.g., from PRRSV infected versus vaccinated pigs, can be measured very well with this assay. Thus the FMIA can be used to compare which cytokines in blood, and what tissue cells secrete, in response to different viral and peptide stimulants for vaccination and infection trials. Thus this grant has provided a means of getting much deeper phenotypic data by testing not just 2 but 10 different important cytokines in diverse samples (serum, oral fluids, culture supernatants).

The studies supported by this NPB grant established the best practices for using this new multiplex FMIA to quantitate levels of swine immune proteins (cytokines, chemokines). Immune proteins help to predict the intensity and speed of the immune response and thus indicate which pigs will resist, or be more susceptible to, PRRSV infection or be protected as a result of PRRS vaccination. The FMIA measures immune proteins involved in 1) the early, innate immunity [interleukin-1β (IL-1β), IL-8, interferon-α (IFNα), and tumor necrosis factor (TNF)], 2) the late, adaptive anti-viral responses associated with T helper 1 (Th1) immunity (IL-12, IFNγ), 3) the alternative Th2 immunity (IL-4), 4) regulatory immunity (IL-10) and cell migration (CCL2). The interplay between levels and timing of expression of these immune protein helps to predict overall immunity.

We used this FMIA to assess pig immune responses to PRRSV infection. Our results show that the SDSU BARC FMIA, supplemented with the recently developed commercial Procarta FMIA, enabled us to measure levels of important cytokines and chemokines, in serum and oral fluids (OF) from PRRSV infected pigs using samples collected through the NPB supported (#10-056) PRRS Host Genetics Consortium (PHGC). The PHGC6 OF samples revealed a vigorous innate immune response to PRRSV infection, as evidenced by the high levels IL-1 β and IL-8 cytokines; unfortunately the levels of IL-12 and IFNα remained low. The stimulatory effects needed from IL-12 and IFNα cytokines was not sufficient to prevent high viral loads in these pigs (10^6 – 10^7 viral equivalents/ml serum). Nor could it stimulate a better adaptive immune response to infection, thus, the low levels of OF IFNγ. Thus all pigs are impacted by PRRSV infection. Our detailed PHGC serum analyses are now underway at BARC. They should enable us to compare the exact responses of each PHGC pig using the individually collected sera over 10 timepoints. Statistical analysis of those results will reveal whether there are different cytokine responses in PRRS susceptible versus resistant pigs. They will help determine how

viral load influences growth, by comparing responses of sera from pigs with high versus low viral load and growth. It is expected that these studies will highlight the role of cytokine proteins and immune response pathways contributing to different PRRS infection outcomes. Final data will be submitted to the secure, password protected PHGC database http://www.animalgenome.org/lunney/index.php maintained at ISU through NPB grant #10-056. Data is being collected on every PHGC pig and serves as a national resource for further PRRS studies.

As a result of our success with the SDSU BARC FMIA cooperative agreements were established with immune bioassay companies. The technology has been transferred to InVitrogen by SDSU, and samples sent from BARC for their test validation. Beta testing began this winter with SDSU and BARC; the InVitrogen FMIA is expected to be publically available in summer 2012. Our results have also been discussed with Affymetrix/Procarta and BioRad. FMIA test validation samples were sent to both companies from BARC. Interactions with Procarta have resulted in new commercial tests that are already available and expansion of their planned tests. The BioRad tests are expected within a year. Thus the NPB investment has been amplified for swine researchers worldwide who will have validated FMIAs available for their overall disease, vaccine and development work. The current tests provide researchers with tools needed to address pig health and welfare issues. Future availability of a wider range of commercial tests will further expand research options.