

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: June 2008 to November 2009

INSTITUTION OR STATION: South Dakota State University

NC-229 REPRESENTATIVE:

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Other PRINCIPLE LEADERS associated with the projects

Fang, Ying SDSU

Wang, Xiuqing, SDSU

Nelson, Eric, SDSU

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

*Per station for ALL Accomplishment = Maximum 3,000 characters including spaces;
Full NC229 report for ALL Accomplishment = Maximum 30,000 characters):*

This section focuses on intended activities, outputs, and short-term outcomes. The report should also reflect on the items that stakeholders want to know, or want to see. The accomplishments should cover only the current year of the project.

Objective 1. Elucidate the mechanisms of host-pathogen(s) interactions.

Indicate progress in the following areas.

1. Research related to pathogenesis/persistence
2. Research related to virus evolution
3. Research related to mechanisms of transmission
4. Research related to viral Immunity and cross-protection
5. Research related to epidemiology

The nonstructural protein 2 (nsp2) of PRRSV has a role in viral replication and may modulate host immunity. Each of the six identified immunodominant nsp2 B-cell epitopes (ES2 through ES7) was deleted from a Type I PRRSV cDNA infectious clone. Deletion of ES3, ES4, or ES7 allowed the generation of viable virus. The Δ ES3 mutant showed increased cytolytic activity and more vigorous growth kinetics, while Δ ES4 and Δ ES7 mutants displayed decreased cytolytic activity and slower growth kinetics *in vitro*. In a nursery pig model, Δ ES4 and Δ ES7 mutants exhibited attenuated phenotypes and the Δ ES3 mutant produced higher peak viral loads. IL-1 β and TNF- α expression levels were down-regulated in cells stimulated (or infected) with the Δ ES3 mutant. These results suggest that certain regions in nsp2 are non-essential for PRRSV replication and may play an important role in modulation of host immunity *in vivo*.

The PRRSV nsp2 contains an ovarian tumor (OTU) domain. Previous studies demonstrated that the OTU domain has deconjugating activity towards ubiquitinated (Ub) and ISGylated products and consequently inhibits the activation of innate immune pathways. To determine whether the nsp2 antagonist function can be ablated from the virus, we introduced point mutations targeting a protein

region predicted to be at the surface of the OTU domain containing a conserved B-cell epitope. Several mutations generated viable recombinant viruses and current research is directed to determine whether these mutations could impact Ub- and ISG15-deconjugation.

PRRSV infection significantly blocks innate anti-viral interferon responses, suggesting that specific PRRSV proteins have developed mechanisms to modulate the host innate immune response. We determined that the PRRSV nsp1 protein can antagonize beta interferon (IFN- β) responses. In PRRSV infected cells, we detected the presence of nsp1 α and nsp1 β and the cleavage sites between nsp1 α /nsp1 β and nsp1 β /nsp2 were identified. Both nsp1 α and nsp1 β dramatically inhibited IFN- β expression and nsp1 β inhibited nuclear translocation of STAT1 in the JAK-STAT signaling pathway. These results demonstrated that nsp1 β inhibits both interferon synthesis and signaling, while nsp1 α alone strongly inhibits the synthesis of interferon. These findings provide important insights into the mechanisms of how nsp1 contributes to PRRSV pathogenesis and how this may impact future vaccine development strategies.

Objective 2. Understand the ecology and epidemiology of PRRSV and emerging viral diseases of swine.

Indicate progress in the following areas.

1. Research related to pathogenesis/persistence
2. Research related to virus evolution
3. Research related to mechanisms of transmission
4. Research related to viral Immunity and cross-protection
5. Research related to epidemiology

Objective 3. Develop effective and efficient approaches for detection, prevention and control of PRRSV and emerging viral diseases of swine.

Indicate progress in the following areas.

1. Research related to pathogenesis/persistence
2. Research related to virus evolution
3. Research related to mechanisms of transmission

A long-term objective is to provide a PRRSV-free semen supply for artificial insemination so mechanical and anti-viral methods were evaluated to reduce risk of transmission. A unilayer density gradient centrifugation method to purify PRRSV contaminated semen allowed for some "risk reduction" by eliminating PRRSV from 71% of semen samples tested. The antiviral chymostatin inhibited PRRSV infection in-vitro. However, further testing is needed to determine an effective animal dose and evaluate effects on sperm quality parameters.

4. Research related to viral Immunity and cross-protection

A multiplex assay to simultaneously quantify 9 porcine cytokines in serum using Luminex xMap™ technology was developed and optimized to detect innate (IL-1b, IL-6, IL-8, IFN-a, TNF-a); regulatory (IL-10), T helper 1 (Th1) (IL-12, IFN-g) and Th2 (IL-4) cytokines. The assay will be of value in vaccine and challenge studies as well as for determining genetic resistance to PRRSV and immune responses to other swine pathogens.

5. Research related to epidemiology

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

Impact statements (500 characters per statement)

This section focuses on actual or intended potential long-term outcomes and impacts, covering only the current year of the project. The report should also reflect on the items that stakeholders want to know, or want to see. List any grants, contracts, and/or other resources obtained by one or more project members as a result of the project's activities. Include the recipients, funding source, amount awarded and term if applicable.

A multiplex assay to simultaneously quantify 9 porcine cytokines in serum using Luminex xMap™ technology was developed and optimized to detect innate (IL-1b, IL-6, IL-8, IFN-a, TNF-a); regulatory (IL-10), T helper 1 (Th1) (IL-12, IFN-g) and Th2 (IL-4) cytokines. The assay will be of value in vaccine and challenge studies as well as for determining genetic resistance to PRRSV and immune responses to other swine pathogens.

Christopher-Hennings, J., Y. Fang, J. Lunney and E. A. Nelson. Development of a rapid, single tube, multiplex test to simultaneously detect immune parameters (cytokines) induced by PRRSV. (National Pork Board, \$101,107, 2008-2009).

The PRRSV nsp1 protein was determined to antagonize beta interferon (IFN-β) responses. We demonstrated that nsp1β inhibits both interferon synthesis and signaling, while nsp1α alone strongly inhibits the synthesis of interferon. These findings provide important insights into the mechanisms of how nsp1 contributes to PRRSV pathogenesis and how this may impact future vaccine development strategies.

Fang, Y., J. Lunney, J. Christopher-Henning, E. Nelson, A. Young. The role of PRRSV non-structural proteins 1 and 2 in host immunity. (USDA-NRI, \$375,000, 1/1/08-12/31/2010).

D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”

Chen, Z, S. Lawson, Z. Sun, X. Zhou, X. Guan, J. Christopher-Hennings, E. A. Nelson, Y. Fang
Identification of two auto-cleavage products of nonstructural protein 1 (nsp1) in porcine reproductive and respiratory syndrome virus infected cells: nsp1 function as interferon antagonist. *Virology* (accepted).

Chen, Z., X. Zhou, J. K. Lunney, S. Lawson, Z. Sun, E. Brown, J. Christopher-Hennings, D. Knudsen, E. Nelson, Y. Fang. Immunodominant epitopes in nsp2 of porcine reproductive and respiratory syndrome virus are dispensable for replication but play an important role in modulation of host immune response. *J. Gen. Virology* (accepted).

Huang, Y. W., Y. Fang, X. J. Meng. 2009. Identification and characterization of a porcine monocytic cell line supporting porcine reproductive and respiratory syndrome virus (PRRSV) replication and progeny virion production by using an improved DNA-launched PRRSV reverse genetics system. *Virus Res.* 145(1):1-8.

Brown, E., S. Lawson, C. Welbon, M. P. Murtaugh, E. A. Nelson, J. J. Zimmerman, R. R. Rowland, Y. Fang. 2009. Antibody response of nonstructural proteins: implication for diagnostic detection and differentiation of Type I and Type II porcine reproductive and respiratory syndrome virus. *Clinical and Vaccine Immunology.* 16(5):628-35.

Molina, R.M., E.A. Nelson, J. Christopher-Hennings, R. Hesse, R.R. Rowland, J.J. Zimmerman, 2009. Evaluation of the risk of PRRSV transmission via ingestion of muscle from persistently-infected pigs. *Transboundary and Emerging Diseases* 56:1-8.

Molina R, S.-H. Cha, W Chittick, S. Lawson, M.P. Murtaugh, E.A. Nelson, J Christopher-Hennings, K.-J. Yoon, R. Evans, R.R.R. Rowland and J.J. Zimmerman. 2008. Immune response against porcine reproductive and respiratory syndrome virus during acute and chronic infection. *Veterinary Immunology and Immunopathology*.

Molina, R.M., W. Chittick, E.A. Nelson, J. Christopher-Hennings, R.R.R. Rowland and J.J. Zimmerman. 2008. Diagnostic performance of assays for the detection of anti-PRRSV antibodies in porcine muscle transudate ("meat juice") samples. *J. Vet. Diagn. Invest.* 20:735-743.

Spilman, M.S., C. Welbon, E.A. Nelson and T. Dokland. 2009. Cryo-electron tomography of porcine reproductive and respiratory syndrome virus (PRRSV): organization of the nucleocapsid. *J. Gen. Virol.* 90:527-535.

Fang, Y., J. Christopher-Hennings, E. Brown, H. Liu, Z. Chen, S. Lawson, R. Breen, T. Clement, X. Gao, J. Bao, D. Knudsen, R. Daly and E.A. Nelson. 2008. Development of genetic markers in the non-structural protein 2 region of a US type 1 porcine reproductive and respiratory syndrome virus: implications for future recombinant marker vaccine development. *J. Gen. Virol.* 89:3086-3096.

2) Abstracts or Proceedings

Fang, Y. 2008. Structural and Function of PRRSV nonstructural proteins: where are we at? 2008 PRRSV Symp.

Reister L, T Clement, E Nelson, J Christopher-Hennings. 2008. Potential mechanical and antiviral methods to insure PRRSV free semen. CRWAD & 2008 PRRSV Symp.

Chen Z, X Zhou, S Lawson, E Brown, R Breen J. Christopher-Hennings, E Nelson, Y Fang. 2008. Expression of foreign proteins in replicase gene regions of porcine reproductive and respiratory syndrome virus. CRWAD & 2008 PRRSV Symp.

Chen Z, X Zhou, D Kuhar, S Lawson, J Lunney, Y Fang. 2008. Effect of PRRSV nsp2 epitope deletion mutants on the induction of cytokine response in porcine alveolar macrophages. CRWAD & 2008 PRRSV Symp.

Xiuqing Wang, Hanmo Zhang, Xueshui Guo. 2009. The interaction between PRRSV and type I interferon induction signaling pathways. 28th American Society for Virology.

Cite authors, year, title, meeting (use abbreviations, e.g., Proc., CRWAD, AASV, 2008 PRRS Symp., etc.) Do not give full dates.

3) Book chapters or monographs

Give full citation

E. FUNDING SOURCES FOR PRRSV RESEARCH

Current

Fang, Y., J. Lunney, J. Christopher-Hennings, E. Nelson, A. Young. The role of PRRSV non-structural proteins 1 and 2 in host immunity. USDA-NRI, \$375,000, 1/08-12/2010.

Meng, X. J., Y. Fang, T. Opriessnig. Innovative approaches to develop a broadly protective and effective vaccine(s) against PRRSV. USDA PRRS CAP2, \$100,000, 1/2009–12/2010.

Christopher-Hennings, J., Y. Fang, J. Lunney and E. A. Nelson. Development of a rapid, single tube, multiplex test to simultaneously detect immune parameters (cytokines) induced by PRRSV. National Pork Board, \$101,107.

Laegreid W., F. Osorio, T. Goldberg, J. Christopher-Hennings, E. Nelson. Immunological consequences of PRRSV Diversity. USDA-NRI, PRRSV CAP2. \$947,885.

Christopher-Hennings J., Y. Fang, E.A. Nelson. Elimination of PRRSV from semen: “On Farm” Mechanical and Antiviral Methods. National Pork Board \$94,558, 2007-2009.

Wang, X. Interaction between PRRSV and interferon alpha/beta induction signaling pathways. USDA NRI 12/2008-11/2010 \$ 100,000.

Fang, Y., J.J. Zimmerman, J. Christopher-Hennings, E.A. Nelson, M. Murtaugh and J. Lunney. Development of diagnostic assays for detecting PRRSV infection using oral fluid samples as an alternative to serum-based assays. National Pork Board. \$119,960.

Fang, Y., W. Zhang, J. Christopher-Hennings, E.A. Nelson and R.B. Baker. Development of an epitope-based vaccine against swine influenza A using a non-toxic enterotoxin as the carrier-adjuvant. National Pork Board. \$49,993.

Lunney, J., J. Christopher-Hennings, E.A. Nelson, Y. Fang, J.P. Steibel and J. Zimmerman. Comparison of early immune responses of pigs which are genetically PRRS resistant/tolerant using a swine-specific immune protein (cytokine) multiplex assay. National Pork Board. \$103,929.

F. WORK PLANNED FOR NEXT YEAR

Major goals for the next year include fulfilling the objectives of currently funded grants. The overall goal of our ongoing USDA-NRI project “The role of non-structural proteins 1 and 2 in host immunity” (Fang, et al.) is to understand the basic functions of PRRSV nsp1 and nsp2 in viral pathogenesis and host immunity. We will further define the roles nsp1 and nsp2 play in the alteration of effective anti-viral innate and cell-mediated immunity. New assays related to PRRSV research priorities will be developed and optimized, including assays using the BioRad Bio-Plex platform. Further evaluation and collaborative utilization of swine cytokine multiplex assays and development of new infectious agent detection assays for oral fluids and other substrates will be priorities. Additionally, we will continue to develop and distribute “shared resources” to the research community, including antibodies, virus isolates, sequences and protocols.