

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: June 2008 to November 2009

INSTITUTION OR STATION: USDA, ARS
National Animal Disease Center
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A. NC-229 REPRESENTATIVE:

2009: Faaberg, Kay; NADC; kay.faaberg@ars.usda.gov

Other PRINCIPLE LEADERS associated with the projects

Kerhli, Marcus Jr.; NADC
Lager, Kelly; NADC
Brockmeier, Susan; NADC
Miller, Laura; NADC
Loving, Crystal; NADC
Neill, John; NADC
John Butler; University of Iowa

B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

Objective 1. Implement a “virtual laboratory” infrastructure through the development and open distribution of resources, materials, protocols, and data among participating researchers.

- a. Miller initiative - Porcine modified Identitag (16bp tags) annotated database has been created with Dr Greg Harhay, USDA, ARS, USMARC, 2009.
- b. Miller initiative - MARC-145 cells provided to: Dr. Moiz Kitabwalla, Lipid Sciences; Dr S. Mark Tompkins, University of Georgia.
- c. Faaberg initiative - MARC-145 cells provided to: Dr. Margo Brinton, Georgia State University; MARC-145, MA-104 and CL2621 cells provided to: Dr. Jens Kuhn, Integrated Research Facility Frederick (IRF-Frederick), NIH/NIAID/DCR
- d. Faaberg initiative - Infectious clone pVR-V7 provided to: Dr. Frederick Leung, Hong Kong University; Dr. Sergey Parinov, The National University of Singapore; Dr. István Kiss, National Veterinary Institute. Infectious clone pIngelvac MLV provided to: Dr. X.J. Meng, Virginia Polytechnic Institute and State University

Objective 2. Achieve biosecurity within herds by preventing the spread of virus within a herd and facilitating its elimination from endemically infected herds.

Faaberg initiative - Nonstructural protein 2 (nsp2), a segment of the PRRSV replicase polyprotein, has been shown to be immunogenic, contains hypervariable segments, encodes a

protease responsible for replicase cleavage and harbors B-cell epitopes. Graduate student Han researched the nature of the PL2 protease, when nsp2 was individually expressed in CHO cells and not associated with virus. He found that the PL2 cysteine protease domain possesses both trans- and cis-cleavage activities, and cleaved only at or near the G|G at nsp2 amino acids 1196|1197|1198. Han then analyzed nsp2 when expressed from the viral genome in MARC-145 cells. He found that nsp2 was now found as at least 6 isomers, all containing the N-termini, but differing in size. He also discovered that heat shock 70kDa protein 5 (HSPA5) was bound to nsp2.

Faaberg initiative - Developed methods to isolate genomic material for full-length nucleic acid sequencing by 454 technology. Using this method, sequenced 15 genomes from a study examining *in vivo* stability after engineering nsp2 deletions. We found that the swine passaged viruses were quite stable, but that the genome acquired more mutations when the length of deletion was maximal. Five other complete PRRSV genomes were also sequenced, four of them representing novel isolates that have not as yet been studied.

Faaberg initiative - Analyzed PRRSV chimeras of attenuated Ingelvac MLV and strain MN184, along with cell-attenuated MN184, and assessed their ability to protect young swine from virulent strain JA142. We found that the previous published ORF1 Chimeras as well as a chimera with a backbone of MLV but with ORFs 5 and 6 of MN184 origin were able to provide some protection. However, the cell-adapted vaccine for MN184 provided equal or greater protection against JA142.

Faaberg initiative - Comparative *in vivo* study of two new isolates along with strains MN184 and SDSU-73. Serum and lavage samples are now being analyzed by virus isolation, qRT-PCR, IFN-gamma, ELISA, and lymphadenopathy.

Kehrli, Faaberg, Miller initiative - Previous results suggested that one section of strain VR-2332 nsp2, when deleted, resulted in virus that did not cause lymphadenopathy when infected into young swine. We are now examining that polypeptide when expressed in adenovirus. The viruses have been made, but no *in vivo* studies have been done.

Butler initiative - Characterization of extreme immune dysregulation (hypergammaglobulinemia, lymphoid adenopathy, immune complex deposition and autoantibodies) following PRRSV infection of gnotobiotic piglets .

Objective 3. Achieve biosecurity among herds by preventing viral spread between sites.

Objective 4. Improve diagnostic assays and create on-farm monitoring systems.

- a. Miller initiative - Established nucleic acid extraction using protocols and quality control testing procedures, with magnetic beads nucleic acid separation, real-time RT-PCR or ELISA detection, for intended animal experiments including mycoplasma, PCV2, BVDV, TTV, PRV and PRRSV

Objective 5. Develop and test PRRSV virus eradication protocols under various ecological settings.

Objective 6. Develop educational outreach tools for disseminating information through established outreach and extension networks to producers, veterinarians, educators, and researchers.

Objective 7. Create an information network to ensure rapid and efficient communication of PRRS virus research protocols and results to scientists, producers, and other stakeholders.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

Title: Global Gene Expression Profiling of PRRSV-infected Alveolar Macrophages

Institution: USDA-ARS-NADC

Investigator(s): John Neill, Laura Miller, Kelly Lager

This study examined the effect of porcine reproductive and respiratory virus (PRRSV) on how genes are expressed in porcine alveolar macrophages (PAMs). PAMs were chosen for this study because they are the primary targets of infection by PRRSV. Serial analysis of gene expression (SAGE) was used here because it allowed us to look at most of the genes expressed in these cells. We determined the normal levels that genes are expressed in normal, non-infected PAMs and then compared this to the gene expression levels in PRRSV-infected PAMs at several time points after infection. It is well established that many pathogens cause changes in expression of specific genes that act to protect the host and clear the infection. This type of response was not seen in these cells. There was surprisingly little in the way of a protective response. Of particular interest was the minimal expression of genes that are involved in attracting other immune cells to the area of the infection. Additionally, there was no response by genes that cause inflammation. This is the first comprehensive study to show the actual breadth of inhibition of an immune response in PAMs by PRRSV. However, the results have also given us tantalizing clues to the mechanism(s) behind this inhibition. There are specific cellular proteins that control the expression of the protective genes and future studies will look at how the virus may be inhibiting their function. This may possibly lead to a means for a producer to intervene that may act to limit or end an active PRRSV infection by restoring the animal's natural protective mechanisms.

Title: Gene Expression in lymph nodes of PRRSV-infected pigs

Institution: USDA-ARS-NADC

Investigator(s): Laura Miller, Greg Harhay, Kelly Lager

The goal of this discovery project is to identify changes that occur in gene expression in porcine lung lymph nodes following PRRSV infection. Knowledge derived from this study will more clearly define the negative effect of PRRSV on the pig immune system, and it may be used to design better cross-protective vaccines. The proposed research is part of an overall plan to evaluate the pig's response to PRRSV infection at the gene expression level. This research is part of two concurrent lines of research at NADC designed to understand how the virus functions, and how the pig responds to a PRRSV infection. Gaining insight into how the virus causes disease may aid development of more cross-protective vaccines that would certainly lead to the production of healthier swine. If more efficacious vaccines were available, then they may lead to strategies to eliminate PRRSV from US swine, a feat that would provide long-term economic impact. The proposed studies are unique because they are comparative in design and complement ongoing studies at NADC. The animal experiment will compare the effect of

PRRSV, SIV, and PCV2 on the pig's immune response. This research will parallel studies at NADC studying effects of these 3 viruses on germ-free pigs, a model system used to determine the direct effect of a virus on the pig, albeit a pig raised in an artificial environment. We suggest the time is right to prioritize the proposed research because technology has advanced to a state where the host response to a virus can be evaluated at a molecular level. In the case of PRRSV, the presumed negative effect can be characterized and compared to the effect of PCV2 and SIV. We believe a better understanding of how each virus might affect swine will provide more insight into how the other viruses function, and how swine try to fight them.

Title: The comparative effect of PRRSV, PCV-2 and SIV on T and B cell levels and phenotype in isolator piglets

Institution: University of Iowa, USDA-ARS-NADC

Investigator(s): Sinkora, M. K.M. Lager, A. Vincent and J.E. Butler

PRRSV produced extreme immune dysregulation characterized by hypergammaglobulinemia, lymphoid adenopathy, immune complex deposition and autoantibodies. 50% of the lymphocytes in the bronchioalveolar lavage (BAL) are B cells and these carry the phenotype of end-stage plasma cells not activated B cells. Unlike SIV-infected piglets that resolve the infection in 20 days, PRRSV infection persists. In parallel with resolution is the appearance of ~15% activated cytotoxic T cells while the numbers of these cells are very low in PRRSV and PCV-2 infected piglets. However the proportion of T cells is 2-4 fold higher in all virus-infected animals than in controls. The hypergammaglobulinemia and elevated level of end-stage B cells appears to represent expansion of the pre-immune repertoire, not an adaptive immune response to PRRSV. This is consistent with the presence of autoantibodies and antibodies to model immunogens that were never administered to the piglets. The list below is a time-line of the major developments after isolator piglets are infected with PRRSV:

1. B cells targeted and driven to end-stage plasma cells
2. Spectratypic analysis and hydrophathy calculations suggest these end-stage cells are from the nondiversified preimmune repertoire (Butler et al., 2007; 2008)
3. Antibodies from these clones recognize 2,4,6-trinitrophenyl (TNP) and many autoantigens without antigen exposure (Lemke et al., 2004)
4. Cytotoxic T cells are absent in the bronchoalveolar lavage
5. Infection persists

Title: Analysis of Nonstructural Protein 2

Institution: USDA-ARS-NADC

Investigator(s): Kay Faaberg

Nonstructural protein 2 (nsp2), a segment of the PRRSV replicase polyprotein, has been shown to be immunogenic, contains hypervariable segments, encodes a protease responsible for replicase cleavage and harbors B-cell epitopes. The nature of the PL2 cysteine protease is an absolutely essential part of the virus and possesses cleavage activities that allow it to work on the replicase polyprotein from which it was derived as well as cleaving other replicase molecules. Thus it has important activities for production of fit virus. In addition, when expressed individually, the protease cleaved only at or near the G|G|G at nsp2 amino acids 1196|1197|1198. We derived viruses with mutations at key sites that allowed partial growth, and these may be utilized in future vaccine formulations. When nsp2 is expressed from the viral genome in MARC-145 cells, at least 6 isomers appeared and all contained the N-termini harboring the PL2

protease, but differed in size and C-terminal cleavage site. He also discovered that cell-derived heat shock 70kDa protein 5 (HSPA5) was associated with nsp2.

Title: PRRSV Genome Analysis by 454 Technology

Institution: USDA-ARS-NADC

Investigator(s): Kay Faaberg

Methods to isolate genomic material for full-length nucleic acid sequencing by 454 technology were developed. Using this method, we concurrently derived full-length genomes for 20 isolates. This method, when published, will allow investigators to sequence many full-length genomes at once, speeding up the time to results as well as lowering the cost for researchers and producers.

Title: Vaccine Efficacy of Porcine Reproductive and Respiratory Syndrome Virus Chimeras

Institution: USDA-ARS-NADC and BI-Vetmedica, Inc.

Investigator(s): J. S. Ellingson, Y. Wang, S. Layton, J. Ciacci-Zanella, M. B. Roof and K. S. Faaberg

We analyzed additional PRRSV chimeras of attenuated Ingelvac MLV and strain MN184, along with cell-attenuated MN184, and assessed their ability to protect young swine from virulent strain JA142. We found that the previous published ORF1 Chimeras as well as a chimera with a backbone of MLV but with ORFs 5 and 6 of MN184 origin were able to provide some protection. However, the cell-adapted vaccine for MN184 provided equal or greater protection against JA142.

D. PRRSV PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed publications

Miller, L.C., Lager, K.M. and Kehrli Jr., M.E. 2009. Effect of porcine reproductive and respiratory syndrome virus infection of porcine alveolar macrophages on Toll-like receptors elicitation of type I interferon responses. Clinical and Vaccine Immunology 16:360-365.

Butler, J.E., P. Weber, N. Wertz and K.M. Lager. 2008. Porcine reproductive and respiratory syndrome virus (PRRSV) subverts development of adaptive immunity by proliferation of germline-encoded B cells with hydrophobic HCDR3s. J. Immunol. 180: 2347-2356

J. Han, Rutherford, M. S., and K. S. Faaberg. 2009. Porcine reproductive and respiratory syndrome virus nsp2 cysteine protease domain possesses both trans- and cis-cleavage activities. J. Virol. 83:9449-9463.

B. R. Tribble, Kerrigan, M., Faaberg, K. S., and R. R.R. Rowland. Identification of an immunodominant region the PCV2 capsid protein recognized by naturally infected and vaccinated pigs. Journal of General Virology, submitted.

J. S. Ellingson, Wang, Y., Layton, S., Ciacci-Zanella, J., Roof, M. B., and Faaberg, K. S. Vaccine efficacy of porcine reproductive and respiratory syndrome virus chimeras. Vaccine, submitted.

Metwally, S., F. Mohamed, T. Burrage, M. Prarat, K. Moran, A. Bracht, G. Mayr, M. Berninger, K. S. Faaberg, L. Koster, L.T. Thanh, V.L. Nguyen, M. Reising, S. Swenson, J. Lubroth and C. Carrillo. Pathogenicity and molecular characterization of emerging porcine reproductive and respiratory syndrome virus in Vietnam 2007. *Transboundary and Emerging Diseases*, submitted.

Vincent, A. L., Lager, K. M., Faaberg, K. S., Harland, M. L., Zanella, E., Ciacci-Zanella, J., Kehrli, Jr., M. E., Janke, B. H., Klimov, A. Susceptibility of Pigs to Pandemic 2009 A/H1N1 Influenza Virus. *Plos Pathogens*, submitted.

Han, J., M. S. Rutherford, and K. S. Faaberg. Proteolytic products of the porcine reproductive and respiratory syndrome virus nsp2 replicase protein. *J. Virol.*, submitted.

Loving, C. L., S. L. Brockmeier, A. L. Vincent, K. M. Lager, and R. E. Sacco. 2008. Differences in clinical disease and immune response of pigs challenged with a high-dose versus low-dose inoculum of porcine reproductive and respiratory syndrome virus. *Viral Immunol.* 21:315-325.

Brockmeier, S. L., K. M. Lager, M. J. Grubman, D. E. Brough, D. ETTYREDDY, R. E. Sacco, P. C. Gauger, C. L. Loving, A. C. Vorwald, M. E. Kehrli, Jr., and H. D. Lehmkuhl. 2009. Adenovirus-mediated expression of interferon-alpha delays viral replication and reduces disease signs in swine challenged with porcine reproductive and respiratory syndrome virus. *Viral Immunol.* 22:173-180.

2) Abstracts or Proceedings

Miller, L.C., Harhay, G.P., Lager, K.M., Kehrli Jr., M.E., Laegreid, W.W. and Neill, J.D. In depth global analysis of gene expression levels in porcine alveolar macrophages following infection with porcine reproductive and respiratory syndrome virus [abstract]. ARK-Genomics Conference 2008: 3rd International Symposium on Animal Functional Genomics, April 7-9, 2008, Edinburgh, U.K. Paper No. ISAFG-P22.p. 38.

Miller, L.C., Chitko-McKown, C.G., Lager, K.M. and Kehrli Jr., M.E. Differential roles of Toll-like receptors in the elicitation of type I interferon responses by alveolar macrophages [abstract]. 2008 International PRRS Symposium, December 5-6, 2008, Chicago, IL.

Chitko-McKown, C.G., Miller, L.C., Lager, K.M. and Kehrli Jr., M.E. Effects of PRRSV infection on TLR-dependent induction of NOS [abstract]. 2008 Conference of Research Workers in Animal Diseases (CRWAD), December 7-9, 2008, Chicago, IL.

Chitko-McKown, C.G., Chapes, S.K., Miller, L.C., and Green, B.T. Characterization of the porcine monocyte-derived cell lines Cdelta2+ and Cdelta2-. 41st Annual Meeting of the Society for Leukocyte Biology, November 6-8, 2008, Denver, CO.

Butler, J.E. 2009 A comparative study of PRRSV, PCV-2 and SIV infections in germfree isolator piglets. Presented at 5th International Veterinary Vaccine Conference, Madison Wisc. July 23.

K. S. Faaberg, J. Han, K. M. Lager, M. E. Kehrli, Jr., and M. S. Rutherford. 2008. PRRSV strain VR-2332 nsp2 deletion mutants attenuate clinical symptoms in swine. XIth International Symposium on Nidoviruses, P43, Oxford, Great Britain.

S. Metwally, C. Carrillo, F. Mohamed, K. Faaberg, M. McIntosh, L. Cox, L. Koster, S. Swenson, T. Burrage, T. Long, T. Beckham, E. Lautner, and J. Lubroth. 2008. Porcine High Fever Disease in Vietnam 2007; PRRS and Other Disease Agents. 51st Annual Meeting of the American Association of Veterinary Laboratory Diagnosticians, Greensboro, NC, USA.

Faaberg, K. S. 2008. State of the Art: Lessons learned through porcine reproductive and respiratory syndrome virus (PRRSV) recombinant technology. International Congress of Virology, 675, Istanbul, Turkey.

Guo, B., Faaberg, K. S., Lager, K., and M. E. Kehrli, Jr. 2009. Genetic stability of PRRSV VR-2332 nsp2 deletion mutants in swine. 28th Annual Meeting of the American Society for Virology, W33-12, Vancouver, Canada.

Loruzzo, A., Faaberg, K. S., Killian, M. L., Koster, L., Vincent, A. L. 2009. One step real-time RT-PCR for 2009 pandemic H1N1 matrix gene detection and quantitation in clinical samples. American Association of Swine Veterinarians 2010 Annual Meeting, March 6-9, 2010, Omaha, NE.

Vincent, A. L., Lager, K. M., Faaberg, K. S., Harland, M. L., Zanella, E., Ciacci-Zanella, J., Kehrli, Jr., M. E., Janke, B. H., Klimov, A. Susceptibility of North American Swine to the Novel A/H1N1 Influenza A Virus. Center of Excellence Symposium, Minneapolis, MN.

3) Book chapters or monographs

E. FUNDING SOURCES FOR PRRSV RESEARCH

1) Current

Miller, Harhay, Lager	NPB	11/01/08-11/01/10	\$139,152
<i>Gene Expression in lymph nodes of PRRSV-infected pigs</i>			
Faaberg, Guo, Miller	NPB	11/01/09-11/01/10	\$53,877
<i>Molecular Identification of Type I Interferon Antagonistic Components of PRRSV Proteins</i>			
Faaberg	NRICGP	10/1/06-6/30/10	\$274,998
<i>Biological Studies of Putative Nonstructural Protein 2 in Porcine Reproductive and Respiratory Syndrome Virus</i>			

F. WORK PLANNED FOR NEXT YEAR

Faaberg:

1. Type I interferon antagonists of PRRSV Proteins
2. Continuing construction of recombinant PRRSV for use as a vaccine
3. In vivo studies of new Type 2 strains

Miller:

1. Analysis of the innate immune response following PRRSV and rPRRSV mutant infection *in vivo*.
2. Assess PRRSV strains that have a reduced capacity for the induction of polyclonal B-cell activation for potential as vaccine candidates
3. Identify significant changes in gene expression during the acute phase of a PRRSV infection in tracheo-bronchial lymph nodes *in vivo*