

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: June 2008 to November 2009

INSTITUTION OR STATION: University of Illinois

A. NC-229 REPRESENTATIVE:

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Other PRINCIPLE LEADERS associated with the projects

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B. PROGRESS OF WORK AND PRINCIPAL ACCOMPLISHMENTS:

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

We analyzed the expression of CD163 on PAMs and macrophages derived from CD14 positive blood monocytes (MDMs), in correlation with PRRSV replication. By flow cytometric analysis, we showed that the levels of CD163 expression correlated well with the overall level of PRRSV replication. We further examined the effects of modulators of macrophage function, including 12-O-tetradecanoylphorbol-13-acetate (TPA), lipopolysaccharide (LPS), and interleukin (IL)-10 on the expression of CD163 and PRRSV replication. Pre-treatment of PAMs or MDMs with TPA or LPS resulted in decreased expression of CD163 and reduction in PRRSV replication. On the contrary, the incubation of CD14 positive monocytes with IL-10 during differentiation into MDMs resulted in up-regulated expression of CD163 with a corresponding increase in PRRSV infection.

Utilizing a yeast two-hybrid screening we identified that the inhibitor of MyoD family-a (I-mfa) domain-containing protein (HIC) is a cellular partner for PRRS virus (PRRSV) N protein. This protein is a homolog of human HIC, a recently identified cellular transcription factor. The specific interaction of PRRSV N with HIC was confirmed in cells by mammalian two-hybrid assay and co-immunoprecipitation and in vitro by GST pull-down assay. HIC is a zinc-binding protein and confocal microscopy demonstrated co-localization of N with the HIC-p40 isomer in the nucleus and nucleolus, and in the cytoplasm with HIC-p32, which is the N-terminal truncation of HIC-p40. The porcine homolog of HIC is universally expressed in pig tissues including alveolar macrophages.

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

See below.

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

Recently, our laboratory developed a porcine alveolar macrophage cell line. This cell line, named ZMAC, was found to efficiently support the replication of a number PRRS virus isolates, often achieving high titers ($>10^7$ TCID₅₀/ml). Given the apparent high permissiveness of ZMAC cells to PRRS virus, we set out to test the proficiency of this cell line to isolate field PRRS virus from clinical samples. The ZMAC line proved highly efficient ($>90\%$) at isolating PRRS virus within 72 hours after exposing ZMAC cells to pig serum samples known to be positive to PRRSV by real-time PCR, from which attempts to isolate PRRS virus in MARC-145, and even primary alveolar macrophages, had failed. Furthermore, the ZMAC cell line allowed us to determine the infectious virus load from these field samples. Remarkably, we observed a significant variation between samples in the ratio of viral genome copy number and titer of infectious virus.

Using an infectious cDNA clone of North American PRRSV strain P129, the viral genome was engineered to transcribe an additional subgenomic RNA initiating between non-structural and structural genes. Two unique restriction sites and a copy of the transcription regulatory sequence for ORF6 (TRS6) were inserted between ORFs 1b and 2a, yielding a general purpose expression vector. The enhanced green fluorescent protein (GFP) gene was cloned between the unique sites such that the inserted gene was transcribed from TRS2 which was located upstream within ORF1b, while the copy of TRS6 drives ORF2a/b transcription. Cells infected with P129-GFP produce virus progeny and showed fluorescence and the inserted gene was phenotypically stable for at least 37 serial in vitro passages. Subsequently, a capsid (C) protein gene was cloned from porcine circovirus type 2 (PCV2) recovered from an outbreak of porcine multisystemic wasting syndrome (PMWS) and inserted into the PRRSV infectious clone vector, generating virus "P129-PCV". Pigs immunized with either P129-GFP or P129-PCV2 produced antibodies specific for GFP or PCV2 capsid respectively.

C. IMPACT AND VALUE OF RESEARCH TO STAKEHOLDERS:

The use of this cell the porcine alveolar macrophage cell line ZMAC to isolate PRRS virus from field samples will enable us to better understand this virus and to development methods to control its spread.

The use of PRRS virus as vector for foreign gene expression is a first, which demonstrates the potential use of PRRSV as a vaccine vector for swine pathogens.

Our studies on the replication of PRRS virus in rodents indicated that PRRSV replication in common laboratory rodent species is inefficient, and suggests that a rodent model for this virus is not appropriate.

The studies on the modulation of CD163 receptor expression and the replication of porcine reproductive and respiratory syndrome virus in porcine macrophages data indicated that the expression of CD163 on macrophages in different microenvironments, in vivo, may determine the replication efficiency and subsequent pathogenicity of PRRS virus.

The interaction of viral capsid with the cellular transcription factor implicates a possible regulation of host cell gene expression by the N protein during PRRSV infection.

D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”

Refereed publications

- 1) Wu, J., J. Li, F. Tian, J. Shi, S. Ren, Z. Lan, X. Zhang, D. Yoo, and J. Wang. 2009. Porcine high fever disease: genetic variation and pathogenicity of porcine reproductive and respiratory syndrome virus in China. *Arch. Virol.* 154: 1579-1588.
- 2) Mohammadi, H., S. Sharif, R.R. Rowland, and D. Yoo. 2009. The lactate dehydrogenase-elevating virus capsid protein is a nuclear-cytoplasmic protein. *Arch. Virol.* 154: 1071-1080.
- 3) Patton, J. B., R.R. Rowland, D. Yoo, and K.C. Chang. 2009. Modulation of CD163 receptor expression and replication of porcine reproductive and respiratory syndrome virus in porcine macrophages. *Virus Res.* 140:161-171.
- 4) Song, C., R. Lu, D. Bienzle, H.C. Liu, and D. Yoo. 2009. Interaction of porcine reproductive and respiratory syndrome virus nucleocapsid protein with the inhibitor of MyoD family-a domain containing protein. *Biol. Chem.* 390: 215-223.
- 5) Du, Y., Zuckermann, FA, and Yoo, D. 2009. Myristoylation of the small envelope protein of porcine reproductive and respiratory syndrome virus is non-essential for virus infectivity but negatively affects its growth. *Virus Res.* (submitted)
- 6) Rosenfeld, P., P.V. Turner, J.I. MacInnes, E. Nagy, and D. Yoo. 2009. Evaluation of porcine reproductive and respiratory syndrome virus replication in the laboratory rodents. *Can. J. Vet. Res.* 73: 313-318.
- 7) Pei, Y., D.C. Hodgins, J. Wu, S.K.W. Welch, J.G. Calvert, G. Li, Y. Du, C. Song, and Yoo, D.. 2009. Porcine reproductive and respiratory syndrome virus as a vector: Immunogenicity of green fluorescent protein and porcine circovirus type-2 capsid expressed from dedicated subgenomic RNAs. *Virology* 389:91-99.
- 8) Vashisht, K., Erlandson, K.R., Firkins, L.D., Zuckermann, F.A., Goldberg, T.L. 2008. Evaluation of contact exposure as a method for acclimatizing growing pigs to porcine reproductive and respiratory syndrome virus. *J Am Vet Med Assoc.* 232:1530-5.

- 9) Vashisht, K., Goldberg, T.L., Husmann, R.J., Schnitzlein, W., Zuckermann, F.A. 2008. Identification of immunodominant T-cell epitopes present in glycoprotein 5 of the North American genotype of porcine reproductive and respiratory syndrome virus. *Vaccine*. 26:4747-53.
- 10) Calzada-Nova, G., Schnitzlein, W., Husmann, R., Zuckermann, F.A. 2009. Characterization of the cytokine and maturation responses. of pure populations of porcine plasmacytoid dendritic cells to porcine viruses and toll-like receptor agonists. *Vet. Imm. Immunopath.* doi: 10.1016/j.vetimm.2009.10.26.

2) Abstracts or Proceedings

1. Yoo, D., Y. Sun, and N. Chen. 2008. One-step mutagenesis of the full-length infectious clone of PRRSV and generation of engineered viruses. Int'l PRRS Symposium, Chicago, IL, Dec 6-7.
2. Hicks, J., D. Yoo, and H.C. Liu. 2008. Identification of domains of PRRS virus GP5 and M proteins that interact with the host Snap-associated protein SNAPIN. Int'l PRRS Symposium, Chicago, IL, Dec 6-7.
3. Wu, J., Li, J., Tian, F., Shi, J., Ren, S., Lan, Z., Zhang, X., Niu, Z., Yoo, D., and Wang, J. 2008. Genetic variation and pathogenicity of porcine reproductive and respiratory syndrome virus in Shandong area of China. Int'l PRRS Symposium, Chicago, IL.
4. Pires-Alves, M., Misra, A., Zuckermann, F.A., Laegreid, W. 2009. Comparison of two cell lines for the propagation of PRRSV. 2009 Int'l PRRS Symposium, Chicago, IL, Dec 6-7.
5. Zuckermann, F.A. , Calzada-Nova, G., Schnitzlein, W., Husmann, R. 2009. Proficient isolation and titration of field PRRS virus from clinical samples using a porcine alveolar macrophage cell line. 2009 Int'l PRRS Symposium, Chicago, IL, Dec 6-7.

3) Book chapters or monographs

None

E. FUNDING SOURCES FOR PRRSV RESEARCH

2008-2009	PI: D. Yoo. Private industry, \$90,000, Antiviral effects of tilmicosin on swine respiratory viruses
2008-2011	PI: D. Yoo. USDA CSREES NRI, \$375,000, Evasion strategies of PRRSV from the host defense
2007-2010	PI: F. Zuckermann. USDA NRI CPG, \$369,064. "In vivo analysis of PRRS virus immunopathogenesis." Tracking Number: GRANT00168948

F. WORK PLANNED FOR NEXT YEAR

For next year we plan to make extensive use of the ZMAC cells line to study the biology of PRRS virus. Studies on the immunobiology of PRRS virus will continue.