

ANNUAL REPORT PROJECT NC-229

PERIOD COVERED: June 2008 to November 2009

INSTITUTION OR STATION:

A. NC-229 REPRESENTATIVE:

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Other PRINCIPLE LEADERS associated with the projects

LeRoith, Tanya, VA Tech
Roberts, P.C., VA Tech
Elankumaran, S., VA Tech
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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

(1). Identification and characterization of a porcine monocytic cell line supporting PRRSV replication and progeny virion production by using an improved DNA-launched PRRSV reverse genetics system. We developed an improved DNA-launched (plasmid DNA transfection-based) reverse genetics system with reduced cost and labor for PRRSV by introduction of ribozyme elements at both termini of the viral genomic cDNA that were placed under the control of a eukaryotic hybrid promoter. The rescue efficacy of PRRSV with this system was approximately 10-50-fold higher than the in vitro-transcribed RNA-based system and the traditional DNA-launched system without the engineered ribozyme elements, as determined by reporter GFP level in transfected cells and the peak titer of the recovery virus. By using this new reverse genetics system, we identified and characterized a porcine monocytic cell line, 3D4/31, capable of supporting PRRSV replication, progeny virion production, and attachment on the cell surface.

2. Research related to virus evolution
3. Research related to mechanisms of transmission

(2). Porcine DC-SIGN: molecular cloning, gene structure, tissue distribution and PRRSV binding characteristics. DC-SIGN, a human C-type lectin, is involved in the transmission of many enveloped viruses. We cloned and characterized the cDNA and gene encoding porcine DC-SIGN (pDC-SIGN). The full-length pDC-SIGN cDNA encodes a type II transmembrane protein of 240 amino acids. Phylogenetic analysis revealed that pDC-SIGN, together with bovine, canis and equine DC-SIGN, are more closely related to mouse SIGNR7 and SIGNR8 than to human DC-SIGN. pDC-SIGN has the same gene structure as bovine, canis DC-SIGN and mouse SIGNR8 with eight exons. pDC-SIGN mRNA expression was detected in pig spleen, thymus, lymph node, lung, bone marrow and muscles. pDC-SIGN protein was found to express on the surface of monocyte-derived macrophages and dendritic cells, alveolar macrophages, lymph node sinusoidal macrophage-like, dendritic-like and endothelial cells but not of monocytes, peripheral blood lymphocytes or lymph node lymphocytes. A BHK cell line stably expressing pDC-SIGN binds to human ICAM-3 and ICAM-2 immunoadhesins in a calcium-dependent manner, and enhances the transmission of PRRSV to target cells *in trans*.

4. Research related to viral Immunity and cross-protection
5. Research related to epidemiology

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
4. Research related to viral Immunity and cross-protection
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.

The establishment of an improved reverse genetic system and the identification of a porcine monocytic cell line supporting PRRSV replication will aid future studies of host-virus interaction of PRRSV.

The identification and characterization porcine DC-SIGN and demonstration of its binding to PRRSV will help better understand the biological role(s) of DC-SIGN family in innate immunity during the evolutionary process.

D. PRRS PUBLICATIONS ISSUED OR “IN PRESS”

1) Refereed publications

Huang YW, Fang Y, Meng XJ. 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.* 2009 Oct;145(1):1-8.

Huang YW, Dryman BA, Li W, Meng XJ. Porcine DC-SIGN: molecular cloning, gene structure, tissue distribution and PRRSV binding characteristics. *Dev Comp Immunol.* 2009 Apr;33(4):464-80.

2) Abstracts or Proceedings

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

Hu J, Meng XJ, and Zhang C. 2008. Purification of native PRRSV virions from cell culture. 2008 PRRS Symposium.

Huang YW, B. A. Dryman, X. J. Meng. 2008. Molecular cloning of porcine DC-SIGN and detection of its potential interaction with porcine reproductive and respiratory syndrome virus. 2008 International PRRS Symposium. 2008 PRRS Symposium.

Huang YW, B. A. Dryman, X. J. Meng. 2008. Molecular cloning of porcine DC-SIGN and detection of its potential interaction with porcine reproductive and respiratory syndrome virus. 2008 International PRRS Symposium. 2008 CRWAD.

3) Book chapters or monographs

Give full citation

E. FUNDING SOURCES FOR PRRSV RESEARCH

1) Current

USDA- CAP-2. Title: Innovative approaches to develop a broadly protective vaccine against porcine reproductive and respiratory syndrome virus (PRRSV).

F. WORK PLANNED FOR NEXT YEAR